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THE DEVELOPMENT OF SCHULTZE DOUBLE FORMATIONS FROM THE EGG OF RANA FUSCA. PART V AND VI

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THE DEVELOPMENT OF SCHULTZE DOUBLE FORMATIONS

FROM THE EGG OF <u>RANA</u> <u>FUSCA</u>. PART V AND VI*

by

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78 Figures in Text

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*Note: For Part I-IV of this work with Figs. 1-44 and Tables VI-XI
see Vol. CXXX (pp. /305-454 of this Journal). The numbering of the
figures in the text of Part V and VI is continued from Part I-IV.

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PART V. ORIGIN OF THE EMBRYONIC ANLAGEN

We now trace the origin of the embryonic anlagen in living   /3
embryos which broadly provides us with a pretty good idea of the
subsequent developmental processes. As mentioned in the Introduc-
tion, Fräulein Wittmann was responsible for investigating the
internal organization of the more or less developed embryos, and
we shall now provisionally report on some of her results.
    In investigating the origin of the embryonic anlagen we
were mainly concerned with the following problems:

1. Is it possible ontogenetically to assign the manifold   /4
Schultze double formations to a single type -- Schultze type of
duplicitas cruciata -- or do ontogenetically fundamentally dif-
ferent types of double formations appear?

2. Can the origin of double formations be explained by
the gastrulation processes described in Part IV?

3. Is there a relation between the form of the double
formation and the outward course of gastrulation?

4. How are the embryonic anlagen oriented to the
original regions of the egg distinguishable before inversion?

5. What is the significance in the origin of double
formations of the region of the original gray crescent which ac-
cording to the results discussed above, retains the same position
in the inverted egg?

6. What potentialities can be ascribed to the different
regions of the inverted egg and what conclusions can be drawn on
the potentialities of the regions of the normal egg?

    We would recall here the comments on the figures (Part I, p.
/329); the key to the embryonic anlagen is the same as in Fig. 1,
unless otherwise indicated in the captions to the figures.
    The embryos to be described are arranged according to the
type of double formation ultimately derived from them. The most
frequent form is the duplicitas cruciata of the Schultze type, the
characteristics of which have already been discussed briefly in

Part I (p. /312 and Fig. 1). As early as 1925 we already concluded from comparison of the most varied stages of development that at least the great majority of all double formations originating from inverted embryos belong ontogenetically to this type of duplicitas cruciata and this has been fully confirmed by study of the developmental processes. Therefore, we shall at first treat the cases of typical duplicitas cruciata and then deal with the modified forms - duplicitas posterior, anterior or lateralis and ventralis and the single formation - derived from an originally laid down duplicitas cruciata or one to be expected from the outward picture of gastrulation. Following this, we discuss the question as to whether there are double formations, which according to their initial ontogenetic anlagen do not belong to the Schultze type of duplicitas cruciata and whether single larvae may develop from inverted eggs, which ontogenetically cannot be classed as double formations at all.

A.   Origin of Typical Duplicitas Cruciata

The duplicitas cruciata of which the whole of Part V, with the exception of sections C and D, is concerned, is that of the Schultze type. If it clearly shows the characteristics given in Part I, p. /312 and in Fig. 1 (two primary anterior ends and two secondary posterior ends) we call it a typical cruciata. It may remain as such up to a later, but in Rana never a very advanced developmental stage, or change into a so-called modified cruciata as in Part V, B. Naturally, it may also wither at an early stage so that we do not then know which of these two paths it would have taken. In Part V, A, we deal with typical cruciatae which remain as such, or die off at an early stage, or are later transformed, although in the latter case we shall not, or only briefly, discuss the transformation itself. A typical duplicitas cruciata may come about in any of the three gastrulation forms described in Part IV and also when an animal blastopore slit appears and it is a truly impressive phenomenon when embryos initially showing exceptional diversity in appearance finally become quite similar double formations of the type indicated. Our discussion will start from the various gastrulation forms. The orientation of the embryonic anlagen to the original main directions of the embryo is very varied; it is determined by the direction of the blastopore anlage insofar as the anlage, as a groove, is oriented. For this reason, in discussing embryos which originate from grooved gastrulation, we shall make a further subdivision corresponding to the direction of the blastopore groove.

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1. Typical Cruciata after Gastrulation in the Form of a
Narrow Groove from the Outset

The origin of a duplicitas cruciata is easiest to understand if the blastopore is a narrow groove from the outset. Such a groove may encircle the embryo or be shorter or even very short; at any rate, it may take a median, frontal or oblique direction.

a) Development from a circular groove

Description of the embryos investigated

Wetzel has described in detail the origin of a double formation from an embryo surrounded by a circular blastopore groove (see Part I, A). Among our test objects which became typical cruciata there were seven embryos with a circular groove all originating from one and the same female (embryo IV 1 and 24-29). Of the embryos of other origin, only one, as far as we could see, formed a circular groove; at any rate, such a blastopore formation was rare in our material. The fact that we observed such a grooved form almost exclusively in the eggs of one female makes it very probable that the properties of the egg varying according to the mother (size ?, the greater or lesser viscosity of its substances?, the quantitative ratio of plasma to yolk?) influence the precise mode of yolk deposition and hence, the form of gastrulation.

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The gastrulation of embryo IV 29 is described in detail in part IV (p. /377 and also in Figs. 11 and 12); its blastopore groove was at an acute angle to the median plane of the egg and showed the peculiar feature of gaping widely at a later stage. Figs. 12e and

e^1 present the embryonic anlagen in the state reached at the time the embryo started to disintegrate. They show very little differentiation, all we can see is that both margins of the groove are bulging, particularly at the original dorsal edge, that is, approximately in the region of the center of the original gray crescent. From our knowledge of all other embryos these two prominent bulges on both sides of the groove represent the rudiments of two heads; but can no longer be identified here as such.

Of the four other embryos of this type which developed these embryonic rudiments, two (IV 1 and 24) had a median circular blastopore groove and the other two (IV 25 and 26) a frontal one; the former gave the two head rudiments precisely in the center of the dorsal edge, i.e., also at the center of the original gray crescent

(Fig. 45 a and b^1) but in the latter two at the left or right edge
(Fig. 46, a and aa). In all other respects these four embryos were
the same as embryo IV 29, described in detail, two of them (IV 1 and
26) were especially like it in that the circular groove gaped widely
at a later stage.
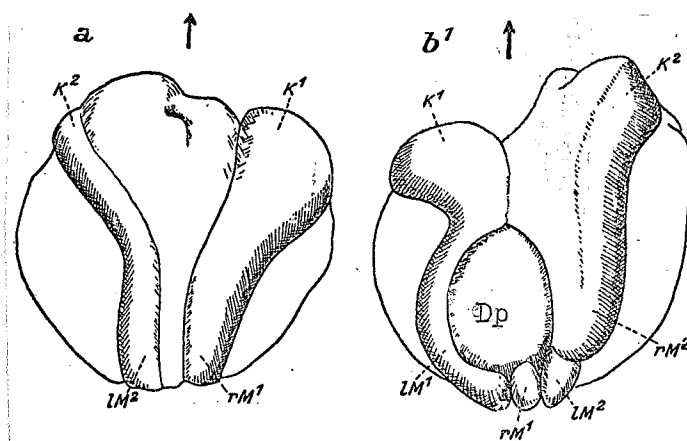
Fig. 45.

IV 1 (a) vegetal view, (b^1) animal view, 3 hr
later.   Dp = yolk plug

The other two embryos (IV 27 and 28) did not manage to develop
their embryonic anlagen, i.e., to be more precise, they did not form
any distinct head rudiments.
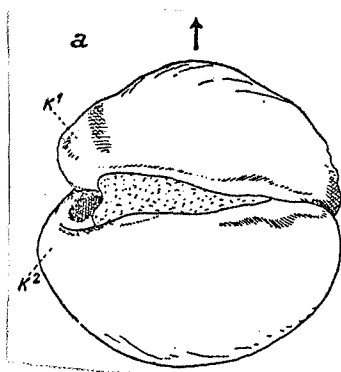
Fig. 46 a.

Embryo IV 26. 25 hr after the start of gastrulation, vegetal view.
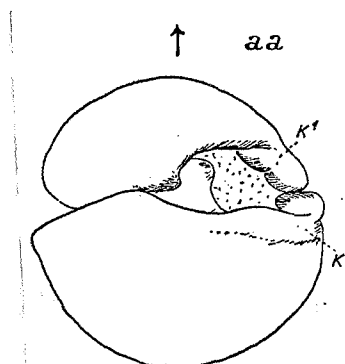
Fig. 46 aa.

Embryo IV 25. 39 hr after start of gastrulation, vegetal view.

SUMMARY

The five embryos which from a circular groove formed embryonic rudiments finally represented a Wetzel form of duplicitas cruciata (see Fig. 1 b): on either side of the blastopore groove an embryonic rudiment developed, which as stated, showed little differentiation. The heads were laid down opposed to each other on both sides of the groove, that is, with a fully median or almost median course of the groove exactly or approximately at the center of the gray crescent (Fig. 47 a and b). As far as we can tell from the slight differentiation of the head anlagen, the anterior end of one head pointed to the right, that of the other, to the left. With a frontal course of the blastopore groove the head rudiments in the cases observed lay at the left or right edge (Fig. 48 a and b), that is, in a region where if the gray crescent was seen at all, it was seen as two horns; the anterior end of one head pointed dorsally, that of the other, ventrally. A neural fold ran from each head along the groove to the right and left seen from the head. Thus (see Part I, p. /312) each embryonic anlage is a spina bifida present as a "yolk plug" in the dorsal cleft of the other embryonic rudiment. We did not in-

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vestigate the internal organization of these double formations; we had hoped, but in vain, to obtain double formations of this type with outwardly considerably more developed embryonic rudiments. However, we do believe we are justified in saying that the embryos described have the same internal structure as described by Wetzel.

If this is so, the lM^1 and rM^2 (also the lM^2 and rM^1 on the everted side) are not joined together but separated by free yolk material: we must assume that under each of the four neural folds there is a chorda. If this is the case, then the following assumption on the origin of these double formations is justified; along the whole length of both margins of the groove the superficial material is involuted so resulting in an archenteric roof along the whole length and on both sides of the groove. Wherever the head rudiments formed, involution was most brisk (Fig. 47 a and 48 a); thus, on both sides of the groove a somewhat more protruding archenteric roof appeared than in the other sections of the groove; this determined the overlying ectoderm for the medullary material of the head section. The direction of involution at the same time determined the direction of the heads.

b) Development from a long groove

In a great number of cases the groove did not encircle the embryo but reached the length of a half meridian or more; we then call it a long groove. It may run median, frontal or oblique to the middle plane of the ovum.

α) Median groove

Description of embryo II 2.

The first ridge ran frontally and a median white band appeared, in the direction of which and in the dorsal part, the groove at first appeared as unconnected small fissures (Fig. 49 a). It then further developed embracing at one end the very white material as the yolk plug and at the other end, somewhat lengthened around the dorsal edge (Fig. 49 b). The stage of its greatest spread over the vegetal and animal surfaces is shown by Figs. 49 c
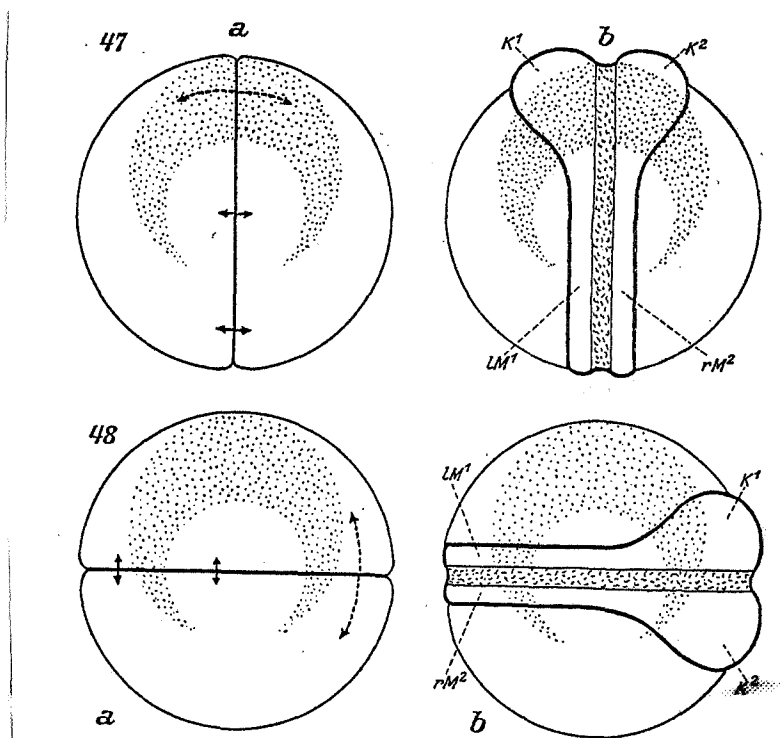
Figs. 47 and 48

Schemes of evolution of a duplicitas cruciata from a circular groove, Wetzel case.

a) circular groove running median or frontally.
b) resulting double formations.

Region of the gray crescent point shaded; embryonic anlagen heavily outlined; K^1 and lM^1, head and left neural fold of one embryonic

rudiment; K^2 and rM^2, head and right neural fold of the second embryonic rudiment; right

neural fold rm^1 of former and left (lM^2) of the latter embryonic rudiments lie in a corresponding manner on the everted side; white yolk shaded; the various long arrows denote involution differing in intensity in the direction of the arrows.

and c^1; the ventral end of the white band can be seen as a small bright spot on the animal surface. Turning inward of the material of the bright band was sketched only in the initial stages in this embryo. However, comparison of Figs. 49 a and b shows clearly
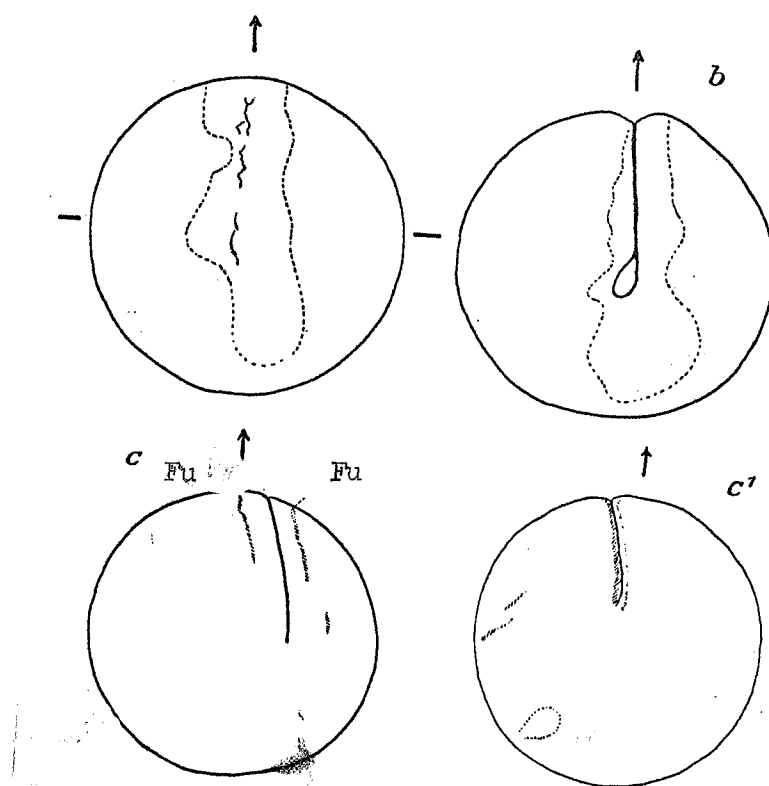
Fig. 49.

Embryo II 2.   a) 12 March, 2.00 pm.   b) 7.45 pm.   c) and

c^1) 13 March, 4.45 am.   Fu = furrows.

that it was mostly involuted in the dorsal region. About 5 hr after the most intense development of the blastopore groove, it had

completely disappeared on the animal surface (Fig. 50 d^1); thus, there is no doubt that it was closed by adhesion of its margins. However, the region it occupied was bounded on both sides by furrows, i.e., on the right and left of this section of the groove the surroundings were bulged out. On the vegetal surface the groove was
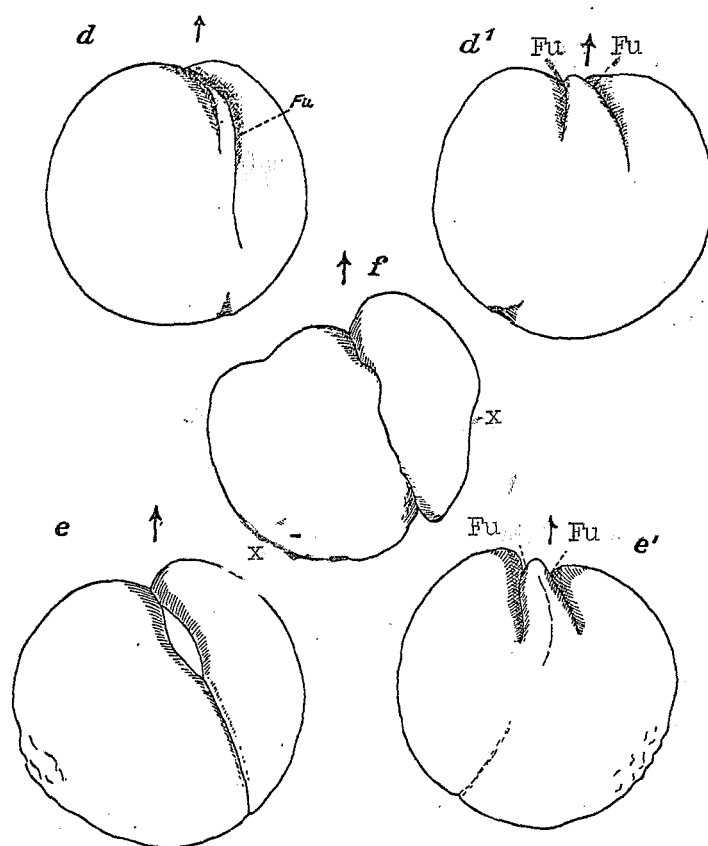
Fig. 50.

Continuation of Fig. 49. d) and d^1) 13 March, 9.45 am.

e) and e^1) 12.15 pm. f) 4.30 pm. Fu = furrows, x = demarcation of head.

at the same time no doubt also starting to close since it was now
considerably shorter and its ventral end was only very weakly
visible (Fig. 50 d). Already in the early stage in Fig. 49 c, the
groove on the vegetal side was enclosed by two furrows marking the
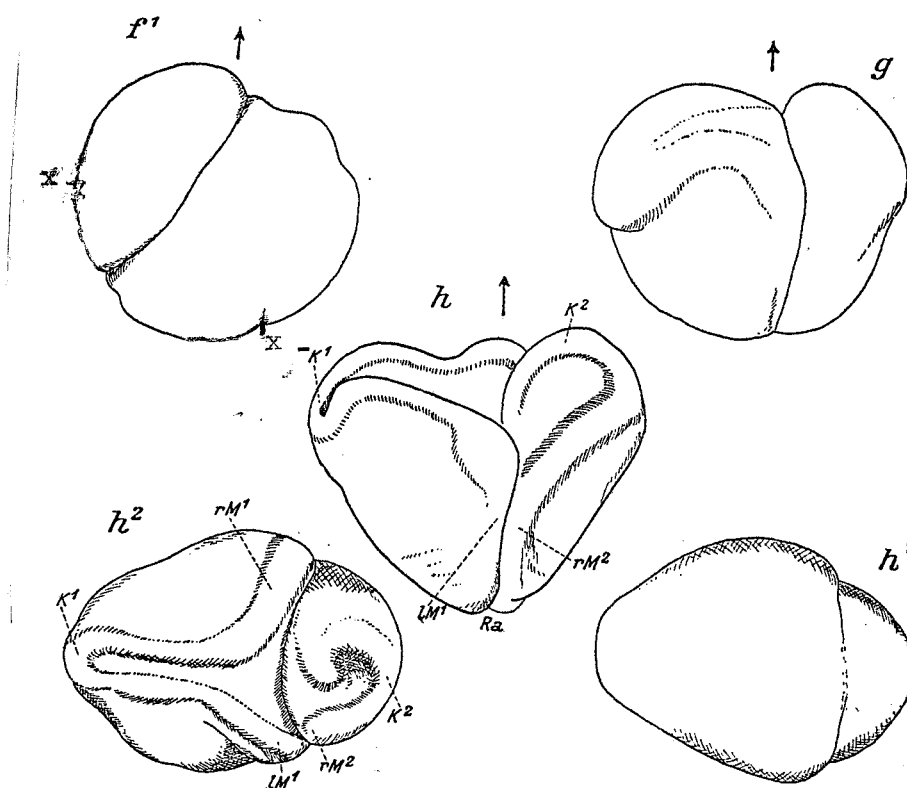
Fig. 51.

Continuation of Fig. 50. f^1) 13 March, 4.30 pm. g) 8.45 pm.
h), h^2) and h^3) 14 March 5.00 am; h^2 dorsal edge view; h^3
ventral edge view.

bulging of the margins of the groove. In Fig. 50 d, the right of these two furrows is still visible and even somewhat longer than before; however, the left is no longer present - or was probably overlooked at the time of sketching owing to the unfavorable direction of the light. At the ventral edge a small indentation appeared presumably related to the bright yolk which had previously occupied this point (compare Fig. 49 c^1). After a further 2-1/2 hr the groove on the vegetal surface was apparently again present and gaping in the proximity of the dorsal edge (Fig. 50 e); it now extended over the whole vegetal surface. It could also be seen on the animal surface although only as a very weakly marked groove (Fig. 50 e^1). Then, it became distinct and deeper all round so that the embryo now appeared to be surrounded by a circular blasto-pore groove (Fig. 50 f and Fig. 51 f^1). Already at this stage we saw the first signs of demarcation of the evaginations of the head as indentations at the edge (x) and some hours later the head rudiments were distinctly visible (Fig. 51 g). They lay to the right and left of the groove in the dorsal region, that is, extended from the region of the center of the original gray crescent right or left. Here too, there was at first more considerable involution of the superficial material to right and left of the margins of the groove.

The final stage of the embryonic anlagen is shown by Fig. 51 h, for the vegetal surface, Fig. 51 h^2 for the dorsal and Fig. 51 h^3 for the ventral side. On the dorsal edge on both sides of the groove which had deepened here, lie the two still scarcely differentiated heads, the smaller one (K^2) turned to the right, the larger one (K^1) to the left*. Right and left neural folds run from each head rudiment; lM^1 and rM^2 have come together to form the secondary vegetal dorsum Ra and rM^1 and lM^2 (not visible in the figures) to

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*What we describe here and in all other cases as heads or head rudiments are in fact, the whole anterior bodies of primary origin, that is heads plus possibly the anterior sections of the dorsi up to the point of bifurcation.

form the secondary animal dorsum Rb (not visible). Both dorsi, i.e., all the neural folds, flatten off towards the ventral edge.

What we saw in Fig. 50 e and e[1] as the reappearance of the blasto-pore groove is now seen to be the neural groove in both dorsi, and at the dorsal edge, the depression between both heads. The gastru-lation groove had, in fact, closed before the appearance of the neural grooves. /13

From the description given there is no doubt about the in-terpretation of the processes: around both margins of the original blastopore groove, the superficial material is turned inwards, es-pecially in the dorsal region, where the groove was first laid down. At the same time, the margins of the groove began to thicken some-what. They adhered along the length of the groove, and only dorsally did a larger gaping gap temporarily appear between them. After closure of the blastopore groove, its original margins became even more elevated so that the neural grooves came into being in both trunks - apparently, reappearance of the blastopore groove. The marked eminence of both head protuberances meant that the path be-tween the neural groove of the animal and the vegetal dorsum now represented a deep depression between both heads*.

Short description of three other embryos

Embryo II 1: the first furrow ran frontally, the bright band and blastopore groove, first laid down at the dorsal edge, were median (Fig. 52 a). The maximum longitudinal development of the blastopore groove on the vegetal and animal surfaces with formation on the latter of a small yolk plug, is shown in Fig. 52 b and

b[1]. It will be clearly seen that the material of the bright band

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*The closure of the blastopore groove, as already discussed in Part IV, preceded the appearance of the neural groove; the former does not (apart from the Wetzel cruciata form) become a neural groove. Earlier (1925) we had accepted the contrary, mistaken, view of Schultze.
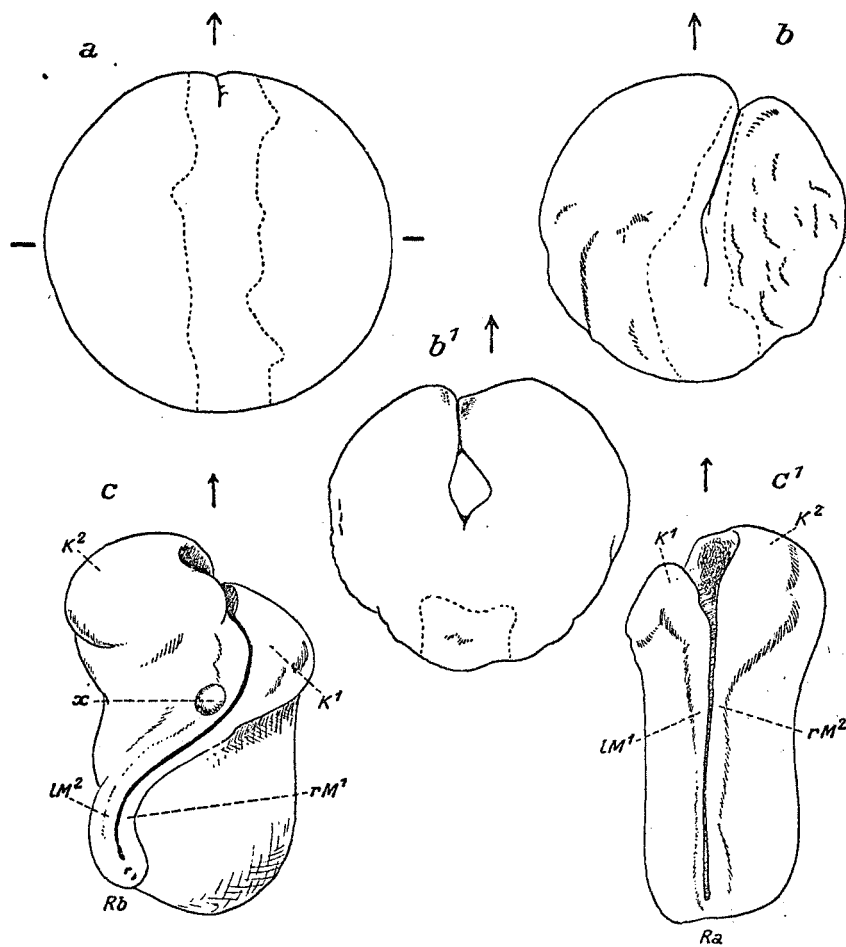
Fig. 52.

Embryo II 1. a) 12 March, 2.30 pm.
b) and b^1) 13 March, 10.15 am. c) and
c^1) 14 March, 12.00 noon. x = sphere
later detached.

is turned inward around the margins of the groove, particularly so
dorsally. On the right side of the embryo many small furrows and
wrinkles appear. The final stage of development is shown in Fig. 52 c
and c^1. It is a typical duplicitas cruciata with long secondary dorsi
and short scarcely differentiated heads. The latter lie to right and
left of the original blastopore groove at the dorsal edge, that is,
roughly in the central region of the original gray crescent and at
the same time at all points where the material had been involuted
most up around the margins of the groove and where the groove first
appeared. A notable feature is the marked lateral curvature of
the vegetal dorsum Rb. This curvature appeared when the wrinkles
and furrows seen in Fig. 52 b had again disappeared. These are most
probably the visible expression of a non-compensated enlargement of
the surface of the embryo due to involution as they became smoother,
the vegetal dorsum shifted and became curved so that a projection
appeared which later separated off as a small sphere (x). We have
mentioned these phenomena since they show that during the develop-
ment of such double formations, surface transformations and shifts
of material occur which usually elude observation. These also include
the longitudinal extension of the whole embryo as can be seen by
comparison of b and c in Fig. 52. This also shows that both dorsi
run closer to the ventral edge of the embryo than did the blastopore
groove at the time of its maximum longitudinal development. Prob-
ably the reason for this is not that the posterior ends of both
dorsi developed at points of the embryo where there was no blasto-
pore groove but rather than the material of the margins of the
groove underwent considerable longitudinal extension. In this
embryo there was no evidence to show that the blastopore groove had
closed before the appearance of the neural groove; this could be
demonstrated only over a short segment of its course.

Embryo I l: gastrulation has been described in detail in
Part IV (p. /371 and also in Figs. 8 and 9). Here, it was clearly
established that the blastopore groove had closed over its entire
length except for a small cleft on the vegetal surface (Fig. 9, i)
before the embryonic rudiments with neural grooves had appeared
(Fig. 53). These were in the form of a typical duplicitas cruciata
with short heads, a long, well-developed vegetal dorsum (Ra) and a
shorter and weaker animal dorsum (Rb). The orientation of the
parts of the double formations to the original regions of the ovum
was the same as in the two cases above. Both heads were also laid
down right and left of the origin of the groove. The embryo re-
mained alive for a longer time and was later fixed as a single
formation (Wittmann).

Embryo I(25): the first furrow ran frontally, a bright band
did not appear but an irregularly outlined bright field did. A

narrow blastopore groove formed in the median direction (Fig. 54, a).
In the stage of its maximum length it embraced more than half the
embryo around the dorsal edge; on the vegetal surface at its ventral

end there was a fairly large yolk plug (Fig. 54, b and b^1). Already
at this stage the blastopore groove was accompanied on both sides by
a furrow. It then closed at the dorsal edge; thereupon, this region
became deeply depressed while on both sides of it the head rudiments
were elevated like beads. On the vegetal and animal surfaces, the
blastopore groove at this time was still distinct, the yolk plug was
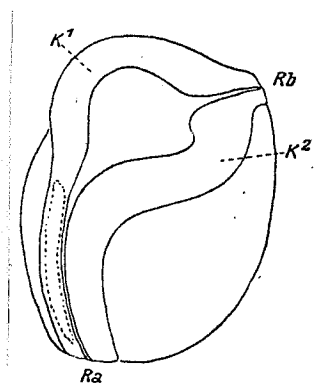
Fig. 53.

Final stage of embryo I 1,
gastrulation of which is de-
picted in Figs. 8 and 9 (Part IV).
Dorsal-right view, somewhat more
vegetal than animal.

sunken flush (Fig. 54, c and c^1). The further course corresponded
on the animal surface to the cases so far described but on the
vegetal surface, the pattern differed. On the former, a strip in
the direction of which the still unclosed part of the blastopore
groove was still identifiable, descended deep between the well-
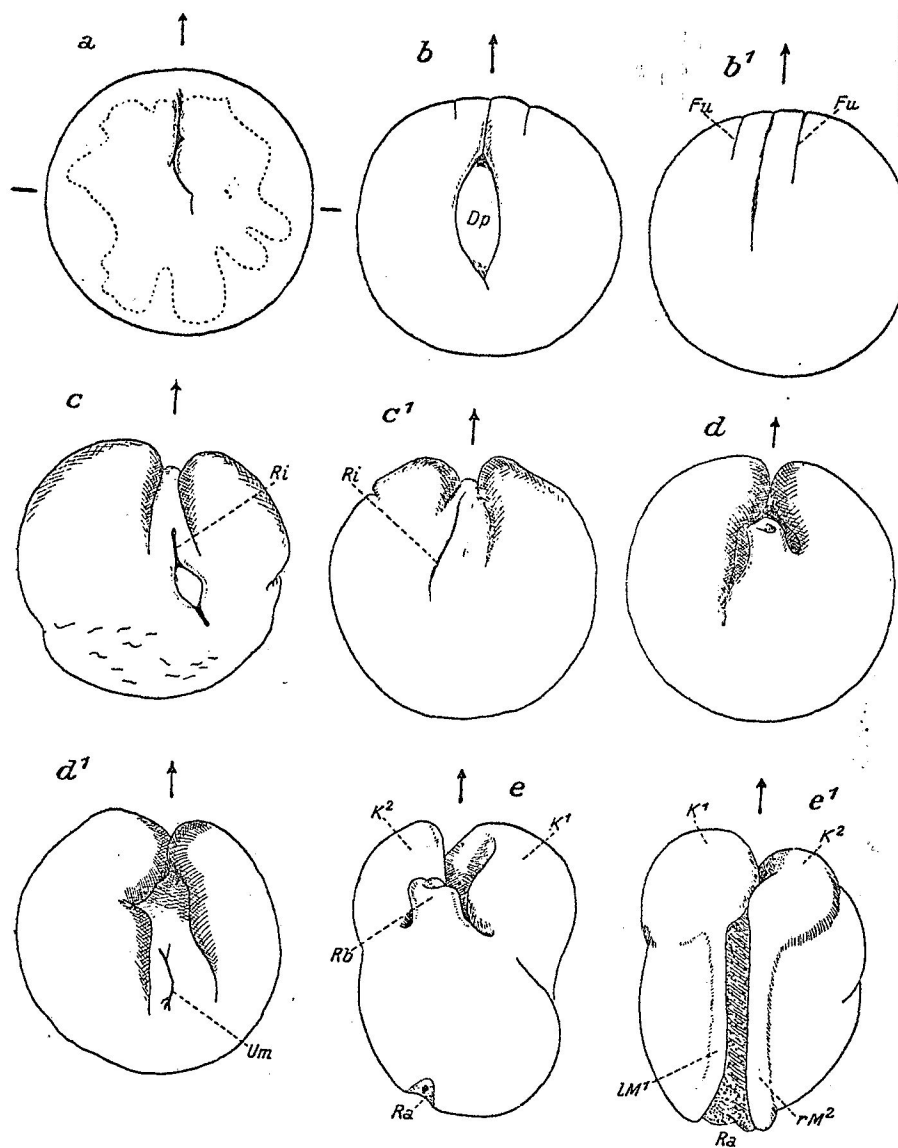
Fig. 54.

Embryo 1. (25) a) 27 March 1925, 1.30 pm; b) and b[1])

28 March 8.30 am. c) and c[1]) 12.00 noon. d) and d[1])

29 March 1.15 am. e) and e[1]) 1.00 pm.

elevated surroundings and became the neural groove (Fig. 54 d[1]).
On the vegetal surface the site at which the yolk plug had sunk be-
came elevated in the form of a sphere and came to lie against the
dorsal edge (Fig. 34, d). At the dorsal edge the depression between
both head rudiments had become very deep. The latest stage at which
the embryo was observed - it was lost on fixation - is shown in

Fig. 54, e and e[1]. The protuberances of the head are unmistakable,
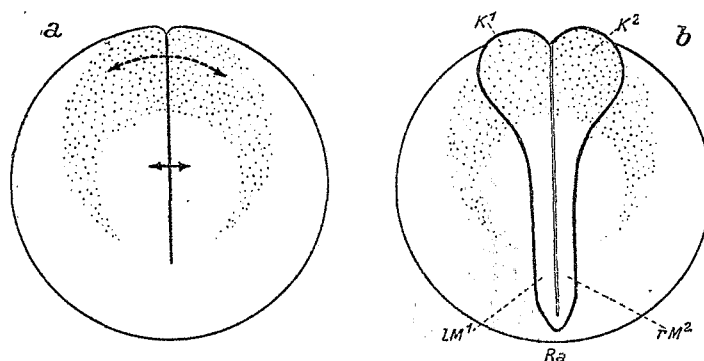
Fig. 55.

Scheme of evolution of a duplicitas
cruciata from a long median groove,
Schultze case. a) long median groove.
b) embryonic rudiments at the stage of
the closed medullary plate in vegetal
view. Gray crescent point shaded, em-
bryonic rudiment heavily outlined. The
various long arrows indicate involution
differing in intensity on the sections
of the groove concerned.

the right one (K[1]) is somewhat larger than the left (K[2]). On the
animal surface a distinctly marked dorsum Ra is present with a broad

neural groove at the bottom of which there is now no trace of the previous blastopore groove and with two neural folds of which one

belongs to K^1 the other to K^2. This dorsum runs in the direction of the original blastopore groove but is considerably longer than this
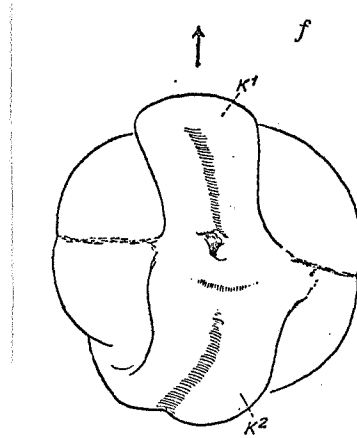
Fig. 56f.

Final stage of embryo XI 2, gas-
trulation of which is shown in
Fig. 13 (Part IV). Vegetal
view.

since it extends beyond the ventral edge to the vegetal surface. This longitudinal growth of the animal dorsum was already hinted at

in the stage in Fig. 54, d^1 and from what we have said above, is based on extension of the cellular material of this dorsum. The vegetal dorsum, Rb, is involuted dorsally and much shorter than the other; its extension was thus obviously less considerable than that of Ra and such that the dorsum first appeared as an upward protu-berance and was then turned inwards anteriorly. This embryo thus behaved in all important respects just like the ones described above but the abberant formation of the vegetal dorsum Rb meant that the

final double formation was no longer completely identical with that
of a typical duplicitas cruciata.

SUMMARY

All four embryos in terms of their mode of genesis fit into
the following scheme (Fig. 55): after the first furrow, a frontal
one, there develops, no matter whether a bright band is present or
not, a median blastopore groove at first laid down dorsally in the
region of the original gray crescent and becoming fairly long
(Fig. 55, a). Around the two margins of the groove both on the
vegetal and on the animal surface, material is turned into the in-
terior, particularly, in the dorsal regions. While the margins
of the groove bulge, the blastopore groove closes more or less
completely over its entire length by adhesion of its margins. A
strip of material having the course of the previous blastopore
groove descended more or less deeply at the dorsal edge with the
result that the head rudiments also appearing in the dorsal region
on both sides of the original groove are separated from each other.
On the vegetal surface the right and left original margins of the
groove become the right and left neural folds of the vegetal
dorsum with the neural groove between them (Fig. 55, b), with
similar development on the animal surface. There thus appears a
typical duplicitas cruciata made up of the two-uniformly laid down

primary heads (K^1 pointing left and K^2 pointing right) and two
secondary dorsi (Ra and Rb - the latter is not shown in the scheme)
formed by fusion of two separate neural folds. The dorsi occupy a
position forming a cross with the median planes of the heads. The
meridian of the blastopore groove describes here, as for the circu-
lar groove, the plane of separation of both individual parts; while
for the circular groove they remain permanently separated by the
open blastopore groove, here as a result of adhesion of the margins
of the groove, there is secondary union of one neural fold of one
embryonic rudiment with the opposite neural fold of the other
embryonic rudiment.

The anterioposterior direction of both individual parts rec-
ognizable from the direction of their heads, is thus turned by 90°
towards the direction of the normal embryonic rudiment.

The development of a double formation is here obviously due

to the same causes as assumed in the case of the circular groove.
Below each margin of the groove involution results in an archenteric
roof, each one of which is directed left and the other right. In-
volution is greatest dorsally, that is, in the region of the gray
crescent. Here, both archenteric roofs are shifted farthest forward,
and so both anterior ends of the archenteron directed towards op-
posite sides appear here as do both head rudiments. Elsewhere in
the groove involution towards both sides is less and in these sec-
tions the neural folds of the dorsi appear.

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According to this interpretation we should find under each
of the two secondary dorsi a single chorda and right and left of
it a series of metameres with both primitive organs and also the
neural tube lying above them originating from two separate halves.
The investigation of such double formations made by Fräulein Wittmann
by sectioning confirmed this expectation although it showed that
under one of the two dorsi, the chorda may show defective develop-
ment or even be entirely absent. Observation of the development
provides reliable evidence showing that the incomplete development
or the lack of these primitive organs is connected with inadequate
processes of involution along the margins of the grooves corres-
ponding to the dorsum in question.

In the formation of the head rudiments and the dorsi the four
embryos described showed differences. The heads (see footnote
on page /12) are usually short in relation to the dorsi but may

also be relatively very long (embryo II 2, Fig. 51, H, K[1]); in the
latter case, the involution in the dorsal region must have been
particularly strong. In this region the degree of involution towards
both sides usually varies,which would explain the different size of
both heads. In fact, examination of sections in the corresponding
gastrulation stages showed that at both sides of the groove there
are usually present archenteric roofs of different size (c.f. Part I-
IV, Plate XI, Figs. 49 and 53, a). Differences in internal organi-
zation of both dorsi have already been discussed above. The fact
that the dorsi may also differ in length must be related to the
difference in length of the sections of the blastopore groove cor-
responding to them and to the difference in extension of the dorsi
themselves. Differences in the surface enlargement of the right and
left sides of the embryo lead to curvature and lateral displacement
of one dorsum (embryo II 1, Fig. 52) and as the result of other ir-
regularities in the extension of the material one dorsum becomes
vertical and moves forward (embryo 1 [25] Fig. 54).

β) Frontal groove

Description of embryo XI 2

     In Fig. 13 the median, first furrow, the frontal white band and the course of gastrulation are described in detail (Part IV,
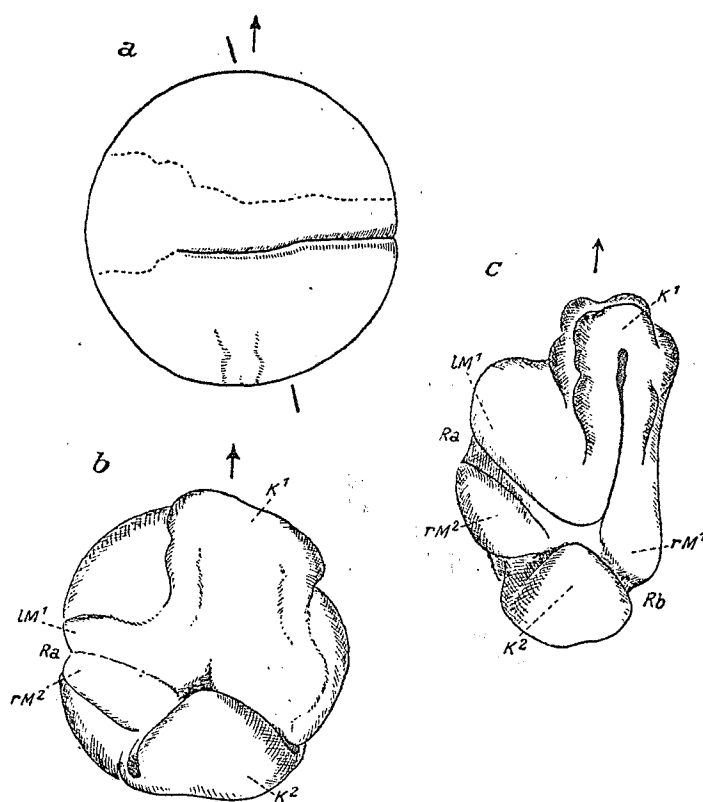
Fig. 57.

Embryo II 4. a) 13 March, 4.45 am;
b) 14 March 4.45 am; c) 2.00 pm.

p. /381). We saw that the frontal blastopore groove began to shorten /20
from both ends, that is, to close, while in its center a deeper de-
pression formed (Fig. 13, e); at this time, numerous wrinkles also
appeared in the ventral region of the embryo. As the description
shows, the blastopore groove must have intersected the region of
the original gray crescent only in the region of its two horns,
whereas its central part runs through the region of the original
vegetal field (Fig. 13, b). Therefore, in this situation the material
turned inward along the dorsal margin of the groove is, in the main,
the material of the gray crescent; however, around the ventral margin
of the groove, at least at its center, only the material of the origin-
al vegetal field could have been involuted. Fig. 56, f, shows the
embryonic rudiments formed. Only both head rudiments are clearly
developed (and the most anterior sections of the dorsi which in this
embryo originally appeared at both sides of the groove perpendicular
to it over a considerable length). One head, K^1, is directed dorsally,
the other somewhat longer, K^2, ventrally. They are separated from
each other by a depression formed at the center of the blastopore
groove (Fig. 13, e). From this point one white, faintly marked groove
radiates to the right and one to the left, obviously both neural
grooves of the secondary dorsi, the margins of which have become
greatly elevated to form the neural folds. Thus in the posterior
secondary trunk sections there is a weakly developed but otherwise
typical duplicitas cruciata. One embryonic rudiment has formed dor-
sally to the blastopore groove, the other ventrally.

Description of two other embryos

Embryo II 4: the first furrow had an almost median course, the
white band and the blastopore groove almost a frontal one. The
latter extended over the greater part of the vegetal surface and on
the right and to some extent also on the animal surface (Fig. 57, a);
later, it extended up to the left margin and also a little around
this margin on the animal surface. The position of the groove in
relation to the region of the original gray crescent is about the
same as in the previous embryo. Fig. 57, b, shows the embryonic /21
rudiments formed. Dorsal to the original groove one has formed
with the large head K^1, ventrally the other with the smaller head
K^2. Branching off towards the left is one of the dorsi Ra, one
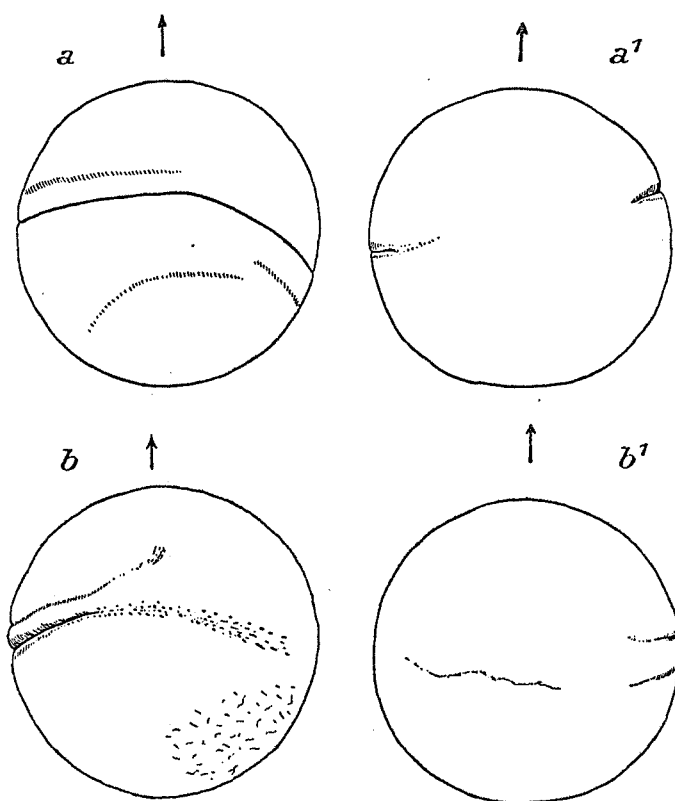
Fig. 58.

Embryo II 3. a) and a^1) 13 March 5.15 am. b)and b^1)
12.45 pm.

neural fold of which lM^1 belongs to head K^1, the other rM^2 to
head K^2. The other dorsum running to the right is hardly visible
in this view and was, in addition, very short. The ends of both
dorsi are joined on the animal side (not shown) by a whitish line
the origin and significance of which as in some other similar cases,

is not known. The neural groove in both dorsi and the whitish
line together encircle the embryo roughly in the direction of the
original blastopore groove. Fig. 57, c, shows the embryo at the
final stage achieved; the right dorsum (Rb) is not better developed
than previously, in contrast to the left one (Ra) and to both heads,
especially the dorsal one (K^1), in which the gill rudiments are
already visible.

/22
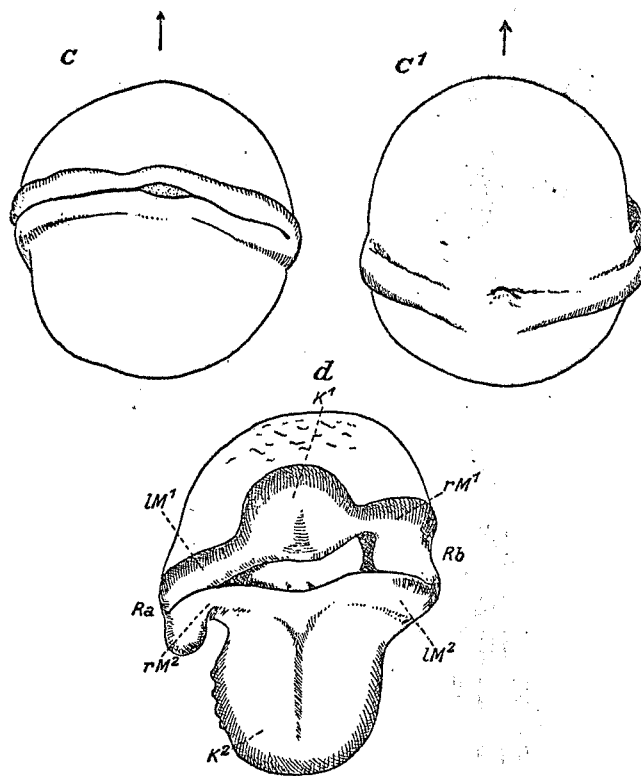
Fig. 59.

Continuation of Fig. 58. c) and c^1) 13 March,
5.00 pm. d) 14 March 5.45 am.

Embryo II 3: the position and direction of the first furrow, the white band and the blastopore groove are about the same as in the previous embryo. The stage of the longest development of the

groove is depicted in Fig. 58, a and a[1]; it occupies roughly the same position in relation to the region of the original gray crescent as in both previous cases. Later, the groove became very indistinct and was undoubtedly closing (Fig. 58, b); its dorsal margin on the left side of the embryo had thickened towards the neural fold which could be traced around the periphery on the animal surface

(Fig. 58, b[1]). On the latter, a very slight depression could be seen

in the direction of the blastopore groove. In Fig. 59, c and c[1] we again see a better developed groove gaping somewhat at the center of the vegetal surface. From the remarks made above (p. /9 and ff. and Figs. 49-51) this groove must be regarded as the neural groove which appeared anew after more or less complete closing of the gastrulation groove and the dorsal and ventral margins of which are distinct neural folds. The latter run around the right and left periphery of the embryo and meet almost at the center of the animal surface. The final stage of development is identical in all essential features to that of the two previous embryos (Fig. 59, d). One embryonic rudiment has formed dorsal to the original groove, the

/23

other ventrally. Both heads, of which one (K[1]), is much smaller

than the other (K[2]), are not greatly differentiated. We cannot say which is the dorsal head and which the ventral, since the relevant details in our drawings are uncertain. It is worth noting that both

trunks, despite their initial considerable length (Fig. 59, c and c[1]) have subsequently become much shorter and the originally small gaping gap in the neural groove (Fig. 59, c) has now become a long and wide hole separating both heads and with white yolk visible at its base.

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SUMMARY

The three embryos described with frontal blastopore grooves also are typical duplicitas cruciatae and are given in diagram form

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in Fig. 60. The remarks made about Fig. 55 amply explain this
diagram but we must stress the important respects in which these
embryos differ from those with a median blastopore groove.
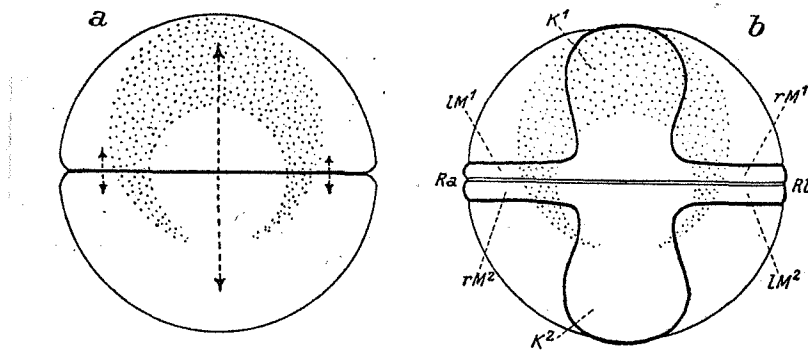
Fig. 60.

Scheme of evolution of a duplicitas cruciata from
long frontal groove, Schultze case. a) long frontal
groove. b) embryonic rudiments at the stage of the
closed medullary plate. Explanations as in Fig. 55.

The plane of the blastopore groove, that is, roughly the
frontal meridian of the ovum, separates both embryonic rudiments.
One developed from the dorsal half of the ovum, the other from the
ventral. The anteroposterior direction of the former is, judging
from its head section, exactly the same as that of a normal em-
bryonic rudiment, that of the latter, precisely the reverse. Thus,
the frontal blastopore groove in these three cases divided the
embryo in such a way that only the two horns of the original gray
crescent fall in the ventral half. Thus, although the middle region
of the gray crescent is absent in the ventral embryonic rudiment,
it may form a well-developed head and two neural folds, the former
being even larger in one embryo (XI 2, Fig. 56, f) than the dorsal
head.

It may be concluded from these facts that in the inverted

embryos the material which lies in the original vegetal field may
involute independently of the middle region of the original gray cres-
cent and then as an archenteric roof, cause the material lying above
to form a medullary plate. However, it is conceivable that an im-
portant factor in this process was that lateral to the material men-
tioned above, there was also material which formed part of the horns
of the gray crescent and influenced the other material in the vegetal
field. However, the evidence of the observations is somewhat against
this; since as follows from the detailed description of the gastrula-
tion of embryo XI 2 (Fig. 13, a-c) involution of the material around
the ventral margin of the blastopore groove occurred mainly at the
center and not laterally where the horns of the gray crescent are
found.

    We did not investigate these embryos in sections; the two
best developed embryos (XI 2 and II 4) died at the end of the ob-
servation, since we wanted to follow up their development to the
extreme limits.

γ) Oblique groove

Description of embryo II 5

    The first furrow ran exactly median, the white band and the
blastopore groove did not run frontally as is usual in such cases
but had a distinctly oblique course (Fig. 61, a). The blastopore

groove at the stage of its maximum length is shown in Fig. 61, b and b^1;
it will be seen that the bright band has considerably narrowed as a
result of turning inward and even almost completely disappeared ven-
tral to the blastopore groove. The latter does not pass through the
center of the vegetal surface but more dorsal, but against this, more
ventral on the animal surface. The outlines of the blastopore groove
shortly before or during the appearance of the embryonic rudiments
were somewhat vague owing to the complicated folding of the surface.

It suffices to discuss the double formation evolved (Fig. 61, c and c^1).
It is a somewhat unusual duplicitas cruciata. One embryonic rudiment
evolved dorsal to the original blastopore groove, the other ventral

to this. The head of the former (K^2) therefore lies wholly dorsally,

that of the other (K^1) on the left side. On the vegetal surface a
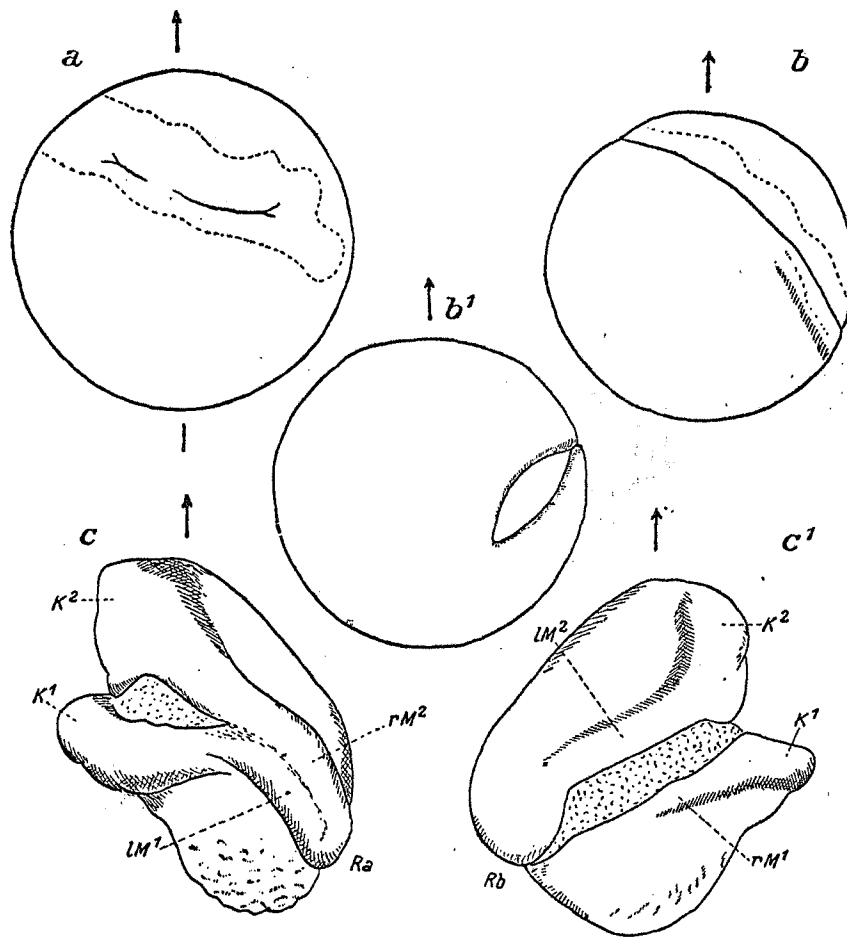
Fig. 61.

Embryo II 5. a) 12 March, 2.00 pm.
b) and b^1) 13 March, 5.00 pm. c) and c^1)
14 March, 5.15 am.

secondary single dorsum (Ra) has formed since both neural folds

(lM^1 and rM^2) have come together as in a typical Schultze duplicitas

cruciata. On the animal surface the two neural folds (rM^1 and

lM^2) have remained separated as in the Wetzel cruciata form, due
apparently, to the subsequent substantial widening of the blastopore
groove. This is clearly related to the fact that on the animal
surface the margins of the groove have enclosed a relatively large
yolk plug.
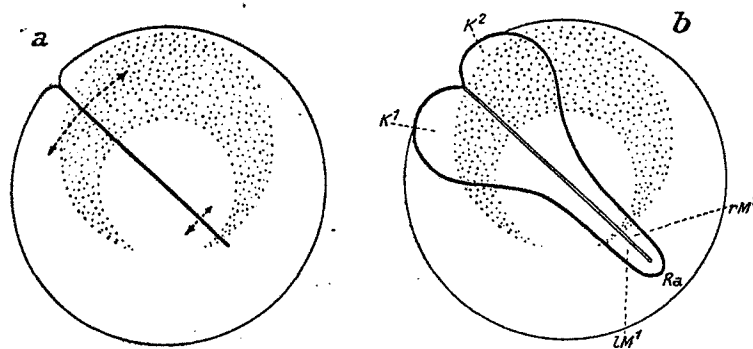
Fig. 62.

Scheme of evolution of a duplicitas cruciata from
long oblique groove, Schultze case. a) long oblique
groove; b) embryonic rudiments at the stage of the
closed medullary plate. Explanations as in Fig. 55.

Short note on three other embryos

The three other embryos (II 6, IV 2 and X 2) displayed in
the most important respects, the same behavior as that described above.
The only point to note is that in one of them (IV 2) the dorsal head
was not larger than the ventral one as was the case for the embryo
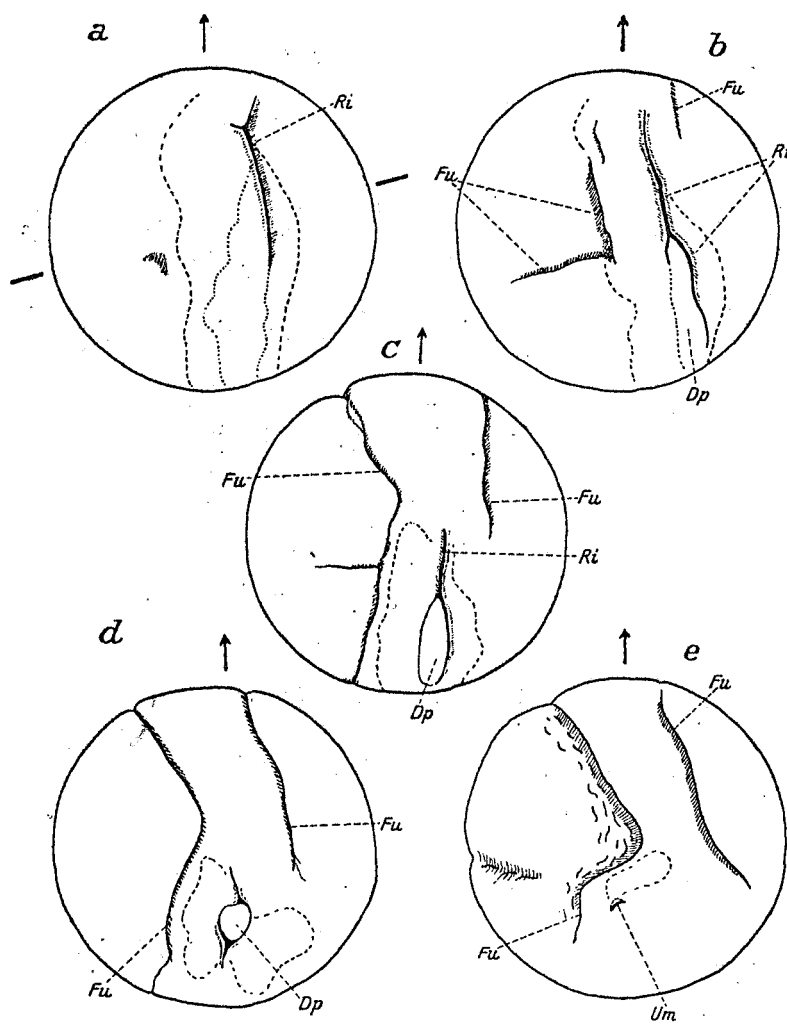
Fig. 63.

Embryo IX 1. a) 1 April, 2.30 pm. b) 8.30 pm.
c) 2 April, 12.45 am. d) 6.30 am. e) 5.30 pm.

dp = yolk plug, Fu = furrow, Ri = blastopore groove,
Um = blastopore residue.

described, but smaller.

SUMMARY

The development of all four embryos with an oblique blastopore groove is schematically depicted in Fig. 62 which after the above remarks, requires no further explanation. For an oblique position of the blastopore groove, one embryonic rudiment is thus formed on each side of the groove. The anterioposterior direction of both judged from their heads, as with the median and frontal course of the blastopore groove lies perpendicular to this, that is obliquely to the anteroposterior direction of a normal embryonic rudiment. One head is formed in the area of the middle region of the original gray crescent, the other which may not necessarily.be the smaller, in the region of one of the two horns of the gray crescent.

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c) Embryos with short groove

We describe as short blastopore grooves, those which do not reach the length of a half meridian and a typical duplicitas cruciata may also evolve from embryos with such a groove. However, such cases are very rare.

Description of embryo IX 1

After the oblique first furrow there appeared a median bright band with a bright white central strip and a median blastopore groove laid down on one side of the bright white strip and more in the dorsal region (Fig. 63, a). This blastopore groove did not reach half the length of the meridian even at the time of its maximum development (Fig. 63, b) and was thus a short one. It closed from the dorsal side towards the ventral side where the bright white material descended into it as a yolk plug (Fig. 63, c and d). At this site its final remains were later seen as a quite small cleft (Fig. 63, e, Um). The blastopore groove from the time of its maximum development

was accompanied on both sides by prominent furrows which themselves
gave the impression of blastopore grooves (Fig. 63, b and c, Fu);
the left furrow put out a side branch towards the left periphery.
This disappeared without trace during the closure of the blastopore
groove so that only the two longitudinal furrows remained (Fig. 63, d).
Later, they were joined (Fig. 63, e) by numerous small wrinkles on
the left side of the embryo. The first signs of the embryonic rudi-

ments can be seen in Fig. 64, f^2; in the direction of the original
blastopore groove there were already hints of a neural ridge; the
two longitudinal furrows pushing each other apart seemed to flank the
neural folds and both head rudiments evolved in the dorsal region.
Since the embryo showed no important changes some seven hours after
sketching of the stage indicated in Fig. 63, e, and was freed from
the fixed position on the plate and subsequently kept in a small
dish, we cannot say with absolute certainty whether both head
rudiments appeared exactly right and left of the origin of the groove
or whether they appeared somewhat beyond its prolongation on the
dorsal edge. However, we may safely assume that the blastopore

rest (Um) still visible in Fig. 64, f^2 was shifted through extension
of the groove material to the ventral edge and perhaps a further
small piece was pushed over to the animal surface. We may therefore
assume that both head rudiments appeared more or less at the point

where the groove was initially laid down. Fig. 64, f^2 does not then
exactly give the vegetal view of the embryo but rather the vegetal
view seen a little from the ventral edge. The double formation can

be clearly seen in Fig. 64, g^2 and g^3. The vegetal-ventral view (g^2)

shows what we may expect to see from Fig. 64, f^2: dorsally, both
heads and from each on both sides of the neural groove, a neural
fold running towards the ventral edge and together forming the second-
ary dorsum Ra. The blastopore groove at no time extended to the animal
surface of the embryo around the dorsal edge; but nevertheless an
unmistakable second dorsum, though much narrower than the vegetal one

appeared on it (Fig. 64, g^3 Rb). We thus have a duplicitas cruciata
even though one dorsum is very rudimentary. The latter vanished
gradually until all that was visible was a small head. Later, the

embryo in every respect looked like a single embryo as in Fig. 64, h^4;
however, it is no longer possible for us to assign accurately the
parts of the embryo, especially, the sucker, to the regions of the
previous double formation. The embryo was fixed at this stage, but

was subsequently lost so that we know nothing about its internal aspects.
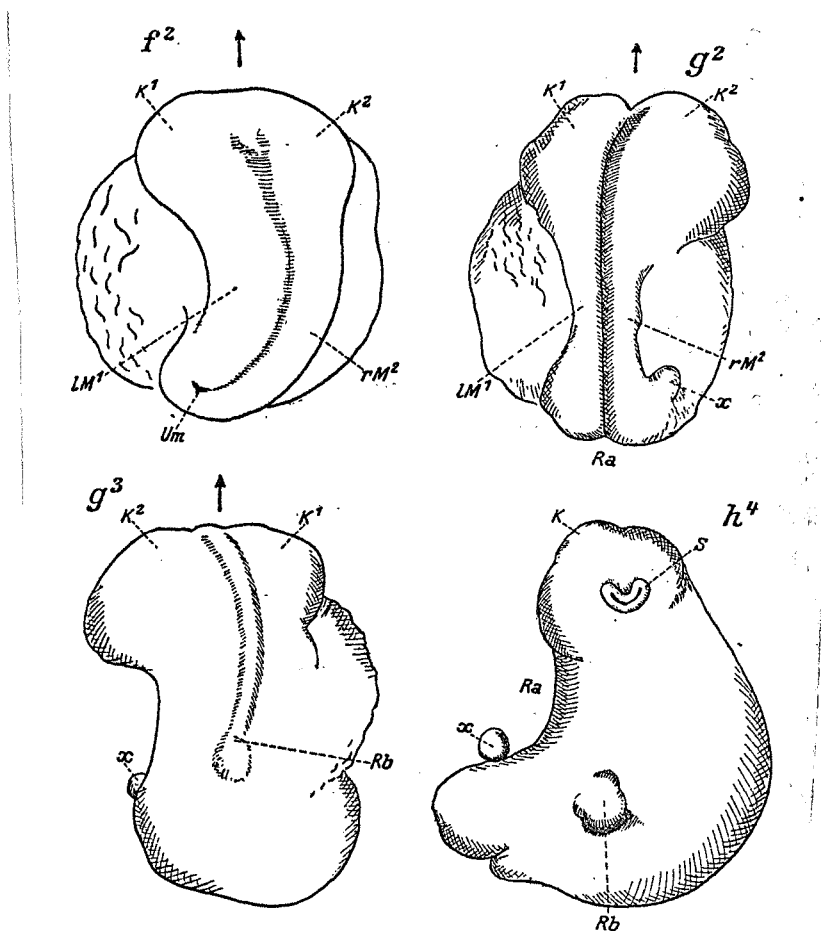
Fig. 64.

Continuation of Fig. 63, f 2. 3 April, 9.00 am; approximate vegetal view, somewhat ventral. g^2) and g^3) 6.00 pm. g^2, same view as f^2; g^3, approximate animal view, somewhat dorsal, h^4) after fixation; probable left-ventral view. S = sucker, x = protuberances.

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A second embryo, 3 (25) behaved in exactly the same manner; it
was fixed at a stage corresponding to Fig. 64, g^2 and g^3. These were
the only two embryos for which one can speak of a short and initially
narrow blastopore groove without at the same time noting complications
in gastrulation and for which analysis was to some extent successful.
Although yet another embryo with a short blastopore groove (II, 7)
also became a duplicitas cruciata the sketches of its developmental
stages are insufficient to define the relation of its individual
parts to the axes of the ovum.

SUMMARY

Had the development in the case of a short blastopore groove
been exactly the same as for a long one, this would have shown up
in the schematic Figure 65: around both margins of the blastopore
groove material turned inward and since the latter was short the
material involuted very far, i.e., two archenteric roofs extending
far into the interior were formed and hence, two long primary
anterior ends (Fig. 65, b) located on both sides of the center of the   /31
original groove. The secondary dorsi forming a cross with the groove
are also short, like the groove; however, they may later lengthen if
their material, as was demonstrated above in several cases, subsequently
stretches in length (Fig. 65, b, broken lines).
However, the development of the embryo described above does
not fit this scheme. Most of the dorsum Ra was laid down over the
segment where the short blastopore groove had been present; the
heads probably evolve entirely at the dorsal end of the groove; and
the dorsum Rb formed on the animal surface where no groove was
present at all. These facts would fit the above scheme if the
material of the blastopore groove after closure extended towards
the dorsal edge and beyond this to the animal surface so that the
animal dorsum Rb could then have formed. Although we do not consider
this assumption very plausible we cannot exclude it. Another ex-
planation is that the short groove was in fact much longer than the
one we saw, extending beyond the dorsal edge to the animal surface.
The possibility that we may have missed this segment of the groove
cannot be completely ruled out particularly since it lay in a quite
darkly pigmented region of the embryo. Other cases still to be
considered provide even more cogent proof that segments of the
blastopore groove cannot be detected. If this is the case, for the

embryo described there would not, in fact, have been a short but a long blastopore groove.

d) Embryos with short groove and an animal blastopore anlage

A special form of gastrulation is that in which together with a more or less short blastopore groove on the vegetal surface, a small blastopore anlage appears on the animal surface. We found four such cases.
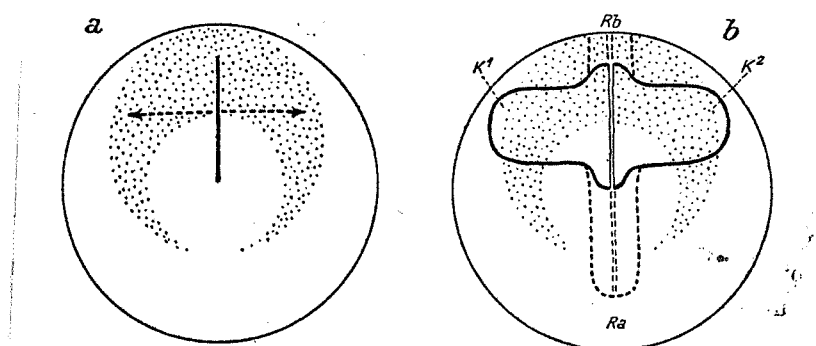
Fig. 65.

Scheme of evolution of duplicitas cruciata from a short groove, Schultze case. a) short groove. b) embryonic rudiments at the stage of the closed medullary plate; Explanations as in Fig. 55.

Description of embryos VI 5-7 and V 1

Embryo VI 5: gastrulation has already been demonstrated in detail in Part IV (p. /385 and Fig. 14). The vegetal blastopore

groove at the stage of its maximum development did not measure half
the length of a median (Fig. 14, b), then gradually closed to form
a small slit (Fig. 14, d) which came to rest on the animal side

through displacement of material (Fig. 14, e A^1). In the meantime,
on the animal side a blastopore anlage, though much shorter, also

appeared (Fig. 14, c^1) and although it was in the median plane, that
is, lay together with the vegetal longer groove in the same median,
it showed a different orientation, namely, was perpendicular to it.
With reduction in size this animal blastopore groove was also shifted,

being roughly in the median plane (Fig. 14, e A^2). At this stage
there were already traces of the embryonic rudiments; their develop-
ment was not traced in detail; but there was formation of a very
typical duplicitas cruciata in which the plane of separation be-
tween the two individual parts corresponds to the meridian in which
both blastopore grooves, the vegetal and the quite short animal
one, were situated. It is fairly certain that both head rudiments
evolved on both sides of the groove at the center of the vegetal
surface originating from both folds which in the stage of Fig. 14,e
were present right and left of the original groove region. The
final stage of the double formation shortly before fixing is shown

in Fig. 66, f^2 and f^3. It is a clearly developed, typical duplicitas
cruciata. The folds mentioned above had risen on the vegetal sur-
face to form typical heads right and left of the original groove;
they were separated by a broad shallow depression in the region of
the groove and the depression in its much narrower part continued
over the ventral and dorsal edge in the direction of the original
groove as neural grooves bounded by neural folds. Of these,

the two ventral ones terminated roughly at the point (Fig. 14, e A^1)
where the ventral end of the blastopore groove was last seen, while
the two dorsal ones terminated in the remains of the cleft mentioned

(Fig. 14, e A^2), which had appeared on the animal surface, without
outwardly showing any connection with the blastopore groove. We can-
not say for this embryo which dorsum is the dorsal one and which the

ventral since although the points denoted by A^1 and A^2 could still
be localized they could no longer be differentiated. The embryo
consists, as shown by subsequent sectional examination (Wittmann),
of two mirror-image almost equal halves both equipped with all the
axial organs. The genesis of these twins is explained by slightly
modifying the scheme given above (Fig. 65). Only the primary head

rudiments are considerably shorter than would be the case for this scheme while the secondary dorsi are considerably longer. For one dorsum which terminated in the remains of the original vegetal blastopore slit we can with reasonable certainty put this down to spread of the material of the groove. For the other dorsum the situation is more complicated in that, on the one hand, it evolved in association with the animal blastopore slit and, on the other hand, from the dorsal end of the vegetal groove.
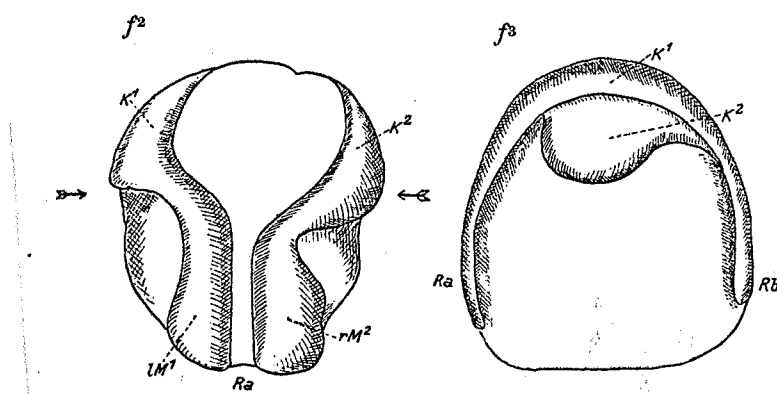
Fig. 66.

Final stage of embryo VI 5, the gastrulation of which is shown in Fig. 14 (Part IV). f^2, vegetal ventral or animal-ventral view. f^3, view in the direction of one of the two arrows in the figure f^2.

Embryo V 1: The vegetal blastopore groove at its maximum stage of development is shown in Fig. 67, a. The right end of the frontal groove denotes the site at which it first appeared. In the same stage, a very short animal blastopore anlage was detectable (Fig. 67, a^1, Sp)., situated in the prolongation of the vegetal

blastopore groove and roughly with the same direction. From this

embryo there evolved a typical duplicitas cruciata which in Fig. 67,b^2 can be seen approximately from the vegetal surface and probably at the same time also, somewhat to the left. The boundary between the more

developed, dorsal head (K^1) and the weaker developed, more lateral

head (K^2) and also the neural groove on the vegetal surface. Both
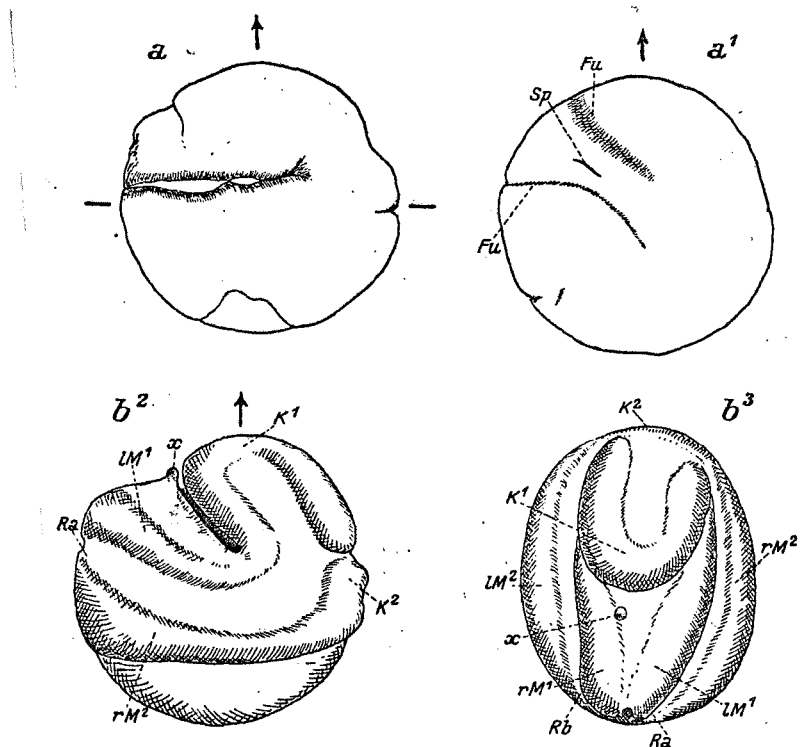
Fig. 67.

Embryo Vl. a) and a^1) 20 March, 3.30 pm. b^2) and b^3) 21 March, 3.15 pm; b^2) vegetal view, somewhat to left; b^3) approximate dorsal view. Fu = furrow, Sp = animal blastopore slit, x = protuberance.

heads, like the above-described embryo IX 1 were laid down on both
sides of the vegetal groove at the site of its first appearance. As
shown by the view of this embryo, roughly from the dorsal side

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(Fig. 67, b^3) an animal dorsum (Rb) is also present, the neural
groove of which runs in the meridian of the animal blastopore an-
lage and so also lies in the prolongation of the vegetal blastopore
groove. About this embryo we can say with reasonably certainty that
the dorsum from the animal side does not lie on any part of the
original vegetal region of the groove. It was evolved only in its
extension and in association with the animal slit.

We describe an embryo of the type shown in Fig. 67, b^2 and b^3
as a "dish-and-lid" embryo (cf. Schleip-Penners, 1925, Fig. 11).

The individual part with the less developed head K^2 ("dish-head")

and the neural folds rM^2 and lM^2 form as it were, a deep basin; on
this like a flat lid sits the other individual part with the more

developed head K^1 ("lid-head") and the neural folds rM^1 and lM^1.

Another embryo, VI 7, behaved during gastrulation in a similar
fashion and also reached the same final stage as embryo Vl. However,
we cannot specify with anything like certainty the localization of
its head rudiments in relation to the origin of the groove on the
vegetal surface. However, one thing is certain, that the plane of
separation of both individual parts again lies in the region and
direction of both blastopore grooves. One posterior end originated
next to the animal blastopore slit, the other next to the blastopore
residue of the vegetal groove.

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Embryo VI 6 also showed on the vegetal surface only a very
short blastopore groove which Fig. 68, a shows at the stage of its
maximum elongation. An even shorter one appeared somewhat later

on the animal surface (Fig. 68, b^1). Both closed to form a short
slit, the animal one in the proximity of the left edge, the vegetal
on the right edge. Again, there developed a very typical duplicitas

cruciata which Fig. 68, c^2 shows in vegetal view but a little to

the right and Fig. 68, c^3, the opposite animal view at the same time,
somewhat to the left. The plane which separated both individual
parts from each other again in this twin formation coincided with
the median in which both blastopore grooves had been present. The

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vegetal dorsum is longer and on stretching curved greatly; the animal dorsum is shorter and remained straight. The depression between the two heads in this embryo is much slighter than elsewhere, and it forms only a narrow slit with no wide notch. Perhaps this is related
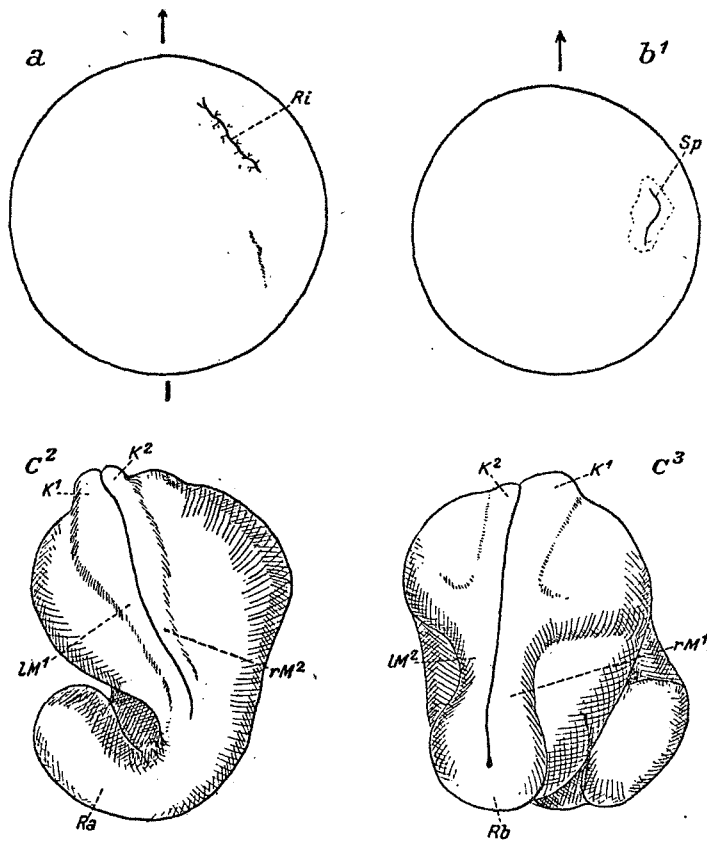
Fig. 68.

Embryo VI 6. a) 24 March, 5.00 am; b^1) 10.00 am.
c^2) and c^3) 26 March, 4.00 pm; c^2) vegetal right
view; c^3) animal-left. Ri = groove,
Sp : animal blastopore slit.

to the favorable further development of this embryo. In fact, it
reached the stage in which in both heads the gill stumps were

distinctly laid down; there were no suckers (Fig. 69, d^4), however.
This was the most fully developed duplicitas cruciata which we ob-
tained for Rana fusca. It lived after this; meanwhile the de-
pression between both heads had levelled out more and more and,
finally, the embryo was fixed as a ventral twin (Wittmann) in
which outwardly there was no longer any indication of the
character of a cruciata.
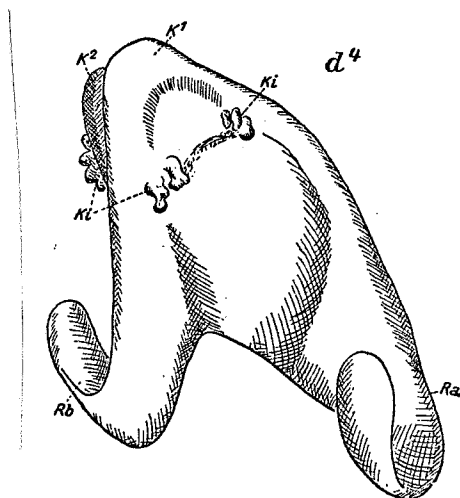
Fig. 69.

Continuation of Fig. 68. d^4) 28 March, 1.00 pm;
view somewhat to left. Ki = gill stumps.

For this embryo too we are not in a position to specify
with certainty the relationship between the origin of the grooves
and the evolution of both head rudiments. However, our findings
and sketches on the development provide some grounds for believing
that as in the case of embryo V 1, the heads evolved at the initial
portion of the vegetal groove. The dorsum Ra was formed above the

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region of the vegetal groove and in its direction. The material concerned underwent considerable extension. The dorsum Rb was laid down in the proximity of the animal slit and in the extension of the vegetal blastopore groove.

SUMMARY

If a duplicitas cruciata evolves from an embryo in which two blastopore anlagen, one vegetal, one animal, appear, then it seems reasonable to suppose that this occurs in the same way as was achieved experimentally by Spemann (1916, 1918, 1919) and Wessel (1926) for Triton (see Figs. 114 and 115). From each of the two blastopores an archenteric roof formed -- pointing somewhat towards the dorsal edge; if during their preliminary growth they come into collision, each is deflected to the right or left so that the entire material of the archenteric roof assumes the form of a cross as also does the medullary material determined by the latter: a duplicitas cruciata forms with two primary single trunks and two secondary single head rudiments forming a cross with the first two.   /37

However, this cannot be the case for the embryos discussed in this section: the archenteric roof material involutes around both margins of the vegetal blastopore groove and does so perpendicular to the margins of the groove and not in the longitudinal direction of the groove. We were not able to establish how involution in the region of the animal blastopore anlage occurs. For embryo VI 5 (Fig. 66) both heads of the duplicitas cruciata lie on both sides of the vegetal blastopore groove and perpendicular to it and at the level of its central section. Thus, it is completely out of the question that the origin of the Janus formation was different from all the cases discussed so far. Thus, in this embryo the relation between the duplicitas cruciata and the animal blastopore anlage apparently only consists in the fact that the animal dorsum extended as far as the site of the animal blastopore anlage. In other respects, its evolution can be readily explained by the scheme in Fig. 65. In the three other embryos mentioned in this section both embryonic rudiments are so arranged as to suggest that the vegetal and animal blastopore anlagen together formed a long blastopore groove on both sides of which one individual part formed each and exactly in the same way as is the case for development from a genuine long groove.

The possibility that the vegetal and animal blastopore grooves together formed a single long groove is all the more likely

in that the very short animal groove always lay fairly precisely in
the direction of the vegetal groove which itself was of somewhat
greater length. Further evidence in support of this view is af-
forded by the shifts in the remains of the blastopore grooves. We
therefore do not rule out the possibility that the appearance of
short blastopore grooves for inverted embryos in some cases is, in
fact, only an apparent one. They are deceptive in that a part of a
long blastopore groove may because of the nature of its development
readily elude observation. Since we could not examine these prob-
lems while the embryos were still alive during the investigation, we

were unable to fix embryos with the features indicated in Fig. 67,a[1]
and to investigate the pattern in sections of the blastopore groove
and the animal groove in particular.

The evolution of a Schultze cruciata from an embryo with a
short vegetal groove and animal blastopore slit is thus not incom-
prehensible but still awaits an adequate clarification.

2. Typical Duplicitas Cruciata after Development of an Initially Broad Blastopore Groove.

It has been shown in section IV, B, that the internal pro-   /38
cesses during gastrulation are essentially the same, whether a nar-
row blastopore groove occurs from the outset, or a broad one is
formed first which may become narrow after descent of the yolk plug.
In both cases, an archenteron, which branches out in two opposite
directions is formed in the interior. Therefore it can be expected,
that we also meet the same or similar conditions as regards the
embryonic anlagen after gastrulation following broad groove forma-
tion, as the case of a narrow groove.

We have analyzed a total of six cases with broad gastrulation
groove up to the appearance of embryonic anlagen, among them those
with long grooves and shorter grooves.

Description of embryo IX 2.

Fig. 70a shows the beginning of gastrulation, which set in,
in an almost completely normal way, dorsally to a bright white complex
on the vegetal surface. However, from this blastopore anlage, which
was at first almost normal, a broad groove developed, running
obliquely from right dorsal to left ventral and enclosing a large

yolk plug between its margins (Fig. 70b); the yolk plug gradually be-
came smaller to the extent that the groove margins moved together
from the right to form a narrow groove (Fig. 70c). Groove closure
then began also from the right. It completely closed, except for a
small gap which persisted for a long time left ventral, and which
gradually shifted in the direction of the groove towards the edge.
It reached the edge when the embryonic anlagen appeared. These are

shown in Fig. 70d^2 in left-dorsal view. A dish-and-lid embryo was

formed, namely one with good dorsal "lid-head" (K^1) and weak ventral

"dish-head" (K^2). Both heads appeared approximately at the right
dorsal edge, hence in the region of the gray crescent and approxi-
mately in the area of the origin of the groove. One dorsum (Ra) was
formed on the vegetal surface above the original groove, after its
closure. The other dorsum (Rb) was formed on the opposite animal
side, even though no sign of gastrulation was observed there.
Examination by sectioning (Wittmann) has shown both dorsi to be
provided with a chorda, but one dorsum Rb is very weakly developed
and parts are present.

　　Two further embryos, the development of which proceeded in
exactly the same way, should be mentioned very briefly. Embryo X1,
as did IX 2, yielded a dish-and-lid embryo. However, in its case
both dorsi, including the one formed on the animal side, even though
no sign of a groove could be observed there during gastrulation, had
quite typical internal axial organs as shown by sectioning (Wittmann).
In the case of another embryo, XI 3, where no groove was observed,
the dorsum formed on the animal surface was very weakly pronounced
from the outset and the embryo was thereafter lost.

Description of embryo X 3.

　　Fig. 71a shows the broad groove in its longest extent on the
vegetal surface. In this stage, a wide yolk plug separates the two
margins of the groove. The margins only approached each other only
at their dorsal end, where the groove had first appeared, and at the
ventral edge. During the further course of gastrulation, the yolk
plug became smaller since, especially at the ventral edge, the groove
margins came more and more together and finally formed a small slit
(Fig. 71b). This narrow groove then extended ventrally towards
roughly the center of the animal surface, forming a narrow slit

(Fig. 71b^1). The last remainder of the yolk plug disappeared then,

and with its disappearance the groove began to close, starting at the center of the surface in a ventral direction (Fig. 71c). Simultaneously, the blastopore groove also started to close from its animal end. Finally, only a small slit remained at the ventral edge (Fig. 72d, Um).
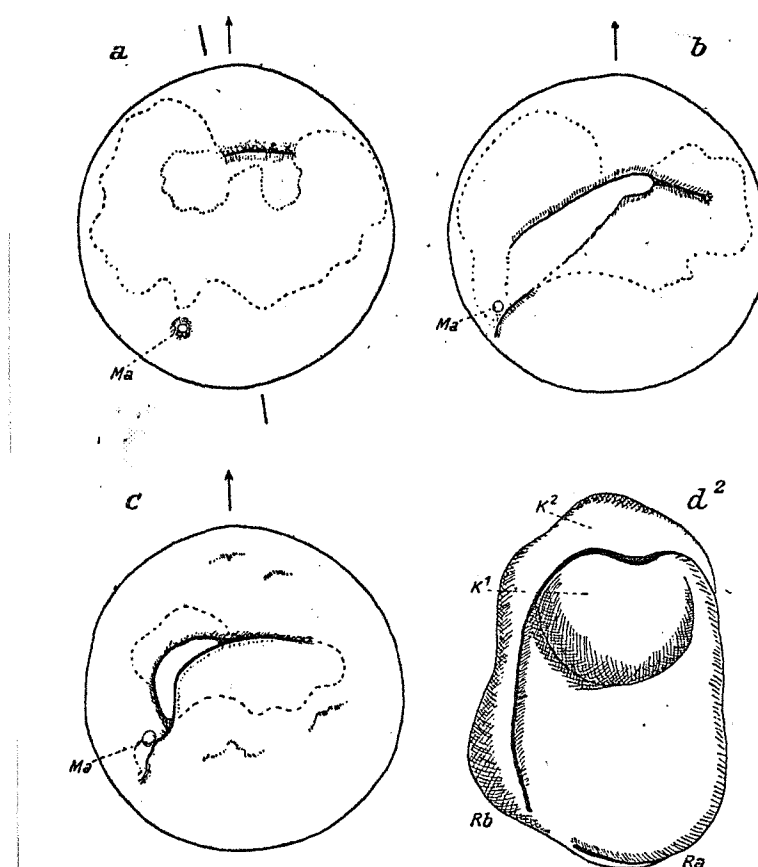
Fig. 70.

Embryo IX 2. a) 1 April, 2.30 pm,
b) 8.15 pm, c) 2 April, 12.30 am.

d^2) after fixation, left-dorsal view.
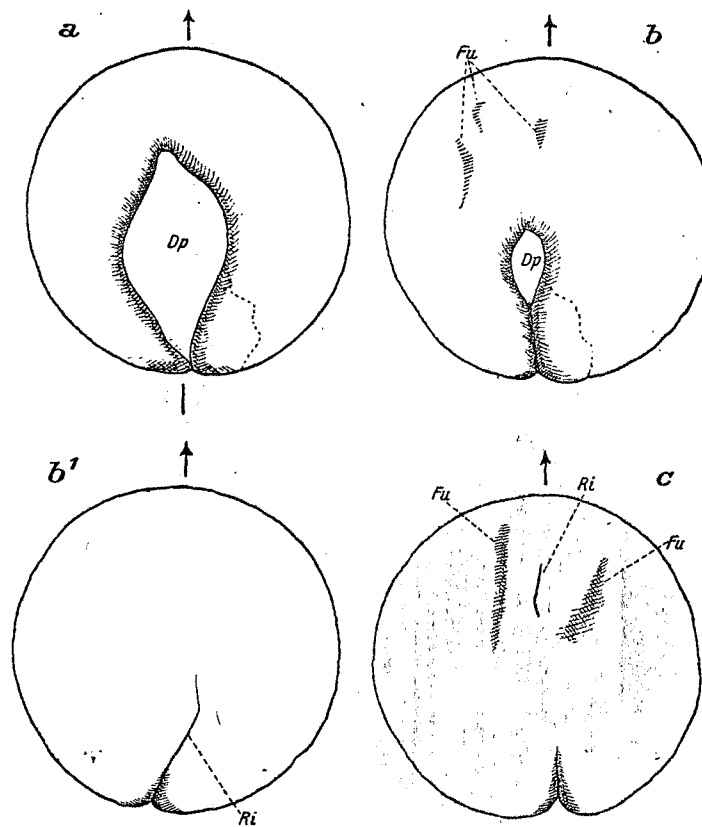
Fig. 71.

Embryo X 3.   a) 1 April, 9.00 pm,

b) and b¹) 2 April, 1.30 am, c)
7.00 am.   Fu = furrow, Ri = blas-
topore groove.

While the groove started to close, after descent of the yolk
plug to the center of the vegetal surface, it also continued in the
dorsal direction towards the edge, starting from the position where
it had at first appeared (Fig. 71c, Ri).   Later on, this continua-
tion reached the edge left dorsally (Fig. 72d, Ri), while the other

part of the groove had closed, except for a small residue at the ventral edge. The secondary continuation of the groove in the dorsal direction had in the meantime again disappeared on the vegetal surface.
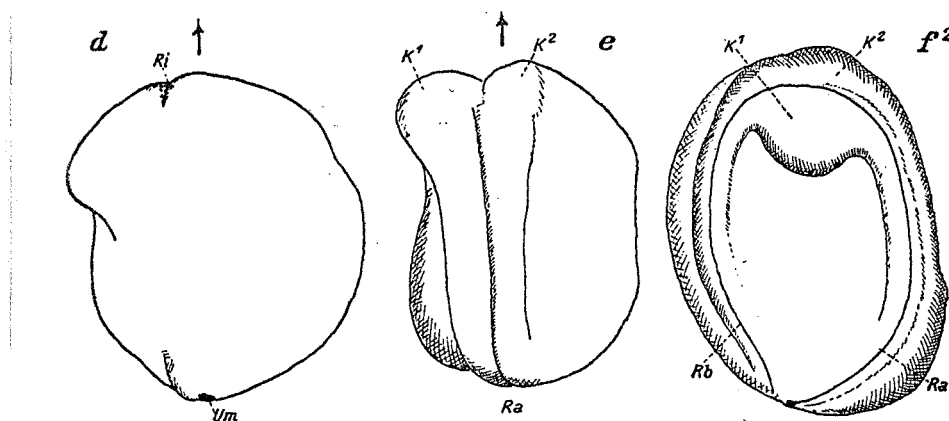
Fig. 72.

Continuation of Fig. 71. d) 2 April,

6.30 pm, e) 3 April, 2.00 am, f^2) 10.00 am. View from the left. Um = blastopore residue.

In the case of this embryo, furrow formations especially dorsal on the vegetal surface had appeared early, (Fig. 71b, Fu). They became still more pronounced (Fig. 71c), when the closing of the groove started after descent of the yolk plug. They then gradually disappeared again (Fig. 72d).

The part of the blastopore groove which had formed secondarily at the dorsal edge, apparently did not close. It later separated the two heads of the twins, which became visible approximately 8 hours after the stage which presented in Fig. 72d (Fig. 72e), where-

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upon, they were seen as typical duplicitas cruciata in the form of a dish-and-lid embryo (Fig. 72f^2). Starting from the blastopore residue at the ventral edge, two clearly formed dorsi extended, one on the vegetal and one on the animal surface in the area of the original groove in the dorsal direction. Both crossed over at the dorsal edge, into two heads, separated from each other by an indentation. One head pointed to the right, the other to the left. From the examination by sectioning (Wittmann) it may be mentioned that both dorsi were provided in almost the same manner with typical axial organs.

Three further embryos, X 4, 5 and 10 behaved similarly and also yielded typical cruciatae. Two of these were lost early, the third could be examined in serial sections and displayed in both dorsi typical axial organs (Wittmann).

SUMMARY

In the first three cases treated (IX 2, X 1 and XI 3) a typical duplicitas cruciata had evolved after formation of an originally broad groove, which narrowed only after descent of the yolk plug. In all three embryos, the groove had an oblique position and was quite long; prior to the appearance of the embryonic anlagen it started to close at the end at which it had first formed and then the closure progressed to the other end. The embryonic anlagen developed in such a way that both heads came to rest at approximately the position of the initial groove end. That is, both heads were situated at right angles to the direction of the original groove. One of the dorsi was located approximately above the original groove area and extended up to the position on the embryo where the groove residue remained. The second dorsum was situated in the opposite direction. It extended from the starting point and in the direction of the groove to the animal surface, where up to that time no signs of a gastrulation groove had been observed. In one of the cases, this animal dorsum was equipped with axial organs as completely as was the one formed above the area of the gastrulation groove; in the two other cases, however, it was more incomplete.

The development of such double formations can be explained in connection with formation of a typical duplicitas cruciata from

a groove, narrow and long from the outset, by assuming that the major involution occurred at both sides of the original position of the groove, perpendicular to its edges (Fig. 73a). As a result, a longer archenteric roof forms there in two opposite directions. It is subjacent to the exterior material and determines the heads (Fig. 73b). Minor involution occurred along the larger remaining part of the groove; where one of the dorsal anlagen was then formed. For the formation of the other, we can assume extension of the groove material at its original end or, which is more probable, that the groove also formed on the edge, but was so faint that we overlooked it. Apparently, the head material generally spread in a more dorsal direction than would correspond to the position of origin of the groove. This is also understandable since, there, the inward turning around the groove edges must have been directed from the outset in a slightly dorsal direction.

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Embryo X 3 and the three similar to it (X4, 5 and 10) do not readily fall within the scheme (Fig. 73). Since in this case, the dorsal end of the broad groove was situated approximately in the center of the vegetal surface (Fig. 71a); it was joined by a transient groove segment, narrow from the outset (Fig. 71c Ri). The head rudiments formed on both sides of the segment. It is not likely that this position of the heads came about because the most brisk involution occurred exactly in this very weakly developed groove setment. It is more probable that also in the case of embryo X3, involution occurred in the manner described schematically in Fig. 73, but that here it must be imagined that the long arrows are directed even more towards the dorsal edge; this would explain why the heads have come to be dorsal to the dorsal end of the originally broad groove segment.

3. Typical Duplicitas Cruciata After Development of an Apparently Normal Blastopore.

We have seen that, independently of the blastopore groove proper forming on the vegetal surface, a gastrulation slip can also occur on the animal side. However, we could never observe it in the presence of a long vegetal groove, but only when the groove proper was short. This suggests some connection between the appearance of the animal slit and the short groove which is found only on the vegetal surface.

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This opinion is further supported by the fact that we also found
often in cases of apparently normal gastrulation, which also only
occurs only on the vegetal surface, a small blastopore slit on the
animal surface, in connection with which the posterior end of a
duplicitas cruciata later usually formed. This animal slit, did not
appear, however,by any means in all cases of apparently normal gastru-
lation.
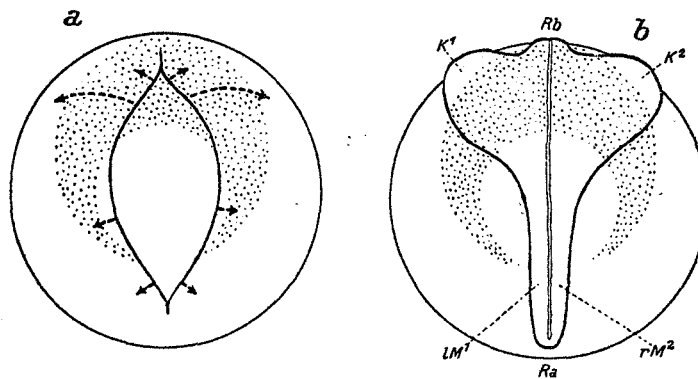
Fig. 73.

Diagram of formation of a duplicitas cruciata
after formation of a broad groove, Schultze's
case.

a) broad groove   b) embryonic rudiments in the
stage of the closed mudullary plate. Explana-
tions as in Fig. 55.

a) Apparently normal blastopore without animal slit.

Description of embryo X 7.

Fig. 74a shows the beginning of gastrulation of this embryo
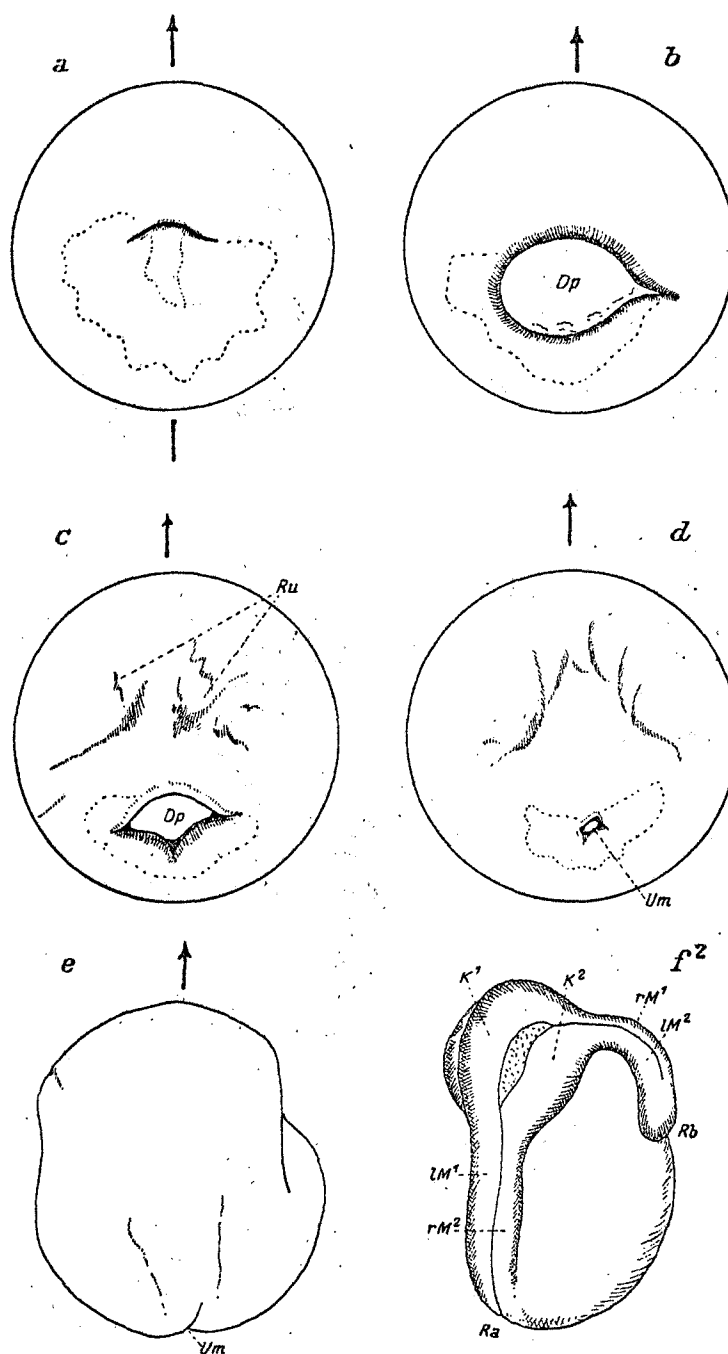
Fig. 74.

Embryo X 7. a) 1 April, 3.15 pm. b) 9.00 pm. c) 2 April, 1.30 am.
d) 6.30 am. e) 3 April, 3.00 am. f²) 10.30 am; vegetal view, slight-
ly right-dorsal. Dp = Yolk plug, Ru = wrinkle, Um = blastopore
residue.

which, apart from having the arc groove situated in the approximate center of the vegetal surface, gave the impression of being normal. Gastrulation proceeded in such a way that the bright material on the vegetal surface was enclosed like a yolk plug (Fig. 74b). However, it should be stressed that the embryo did not present a completely normal picture in this stage. First, the slit branched off to the right of the generally oval-shaped closed blastopore. Furthermore, it should be stressed that the white material laid very deep in contrast to the normal, somewhat projecting, yolk plug. Conversely, the margin of the groove bulged considerably. During the further course of gastrulation, the yolk plug descended at the spot, its ventral part remaining visible the longest (Fig. 74c and d). In the meantime, very pronounced fold- and wrinkle formations appeared in the dorsal part of the vegetal surface and further added to the abnormal appearance of the embryo. But, these fold formations disappeared again at the time when the blastopore residue, in the form of a small slit, had receded to the ventral edge (Fig. 74e, Um). If one considers that the first blastopore slit to become visible had appeared at the center of the vegetal surface and that, now, the last remains of the blastopore were found at the ventral edge, it turns out that the blastopore, as such, traversed the median plane during its closure the distance of the arc of a quarter circle. That is, it covered a definite path of closure.

From this embryo, which, apart from the fold formations mentioned and the pronounced bulging of the edge of the blastopore, showed largely normal gastrulation, a typical duplicitas cruciata nevertheless evolved (Fig. 74 f^2). Its orientation to the original ovum material was in all probability as follows: A long dorsum Ra developed from the blastopore residue, starting at the ventral edge, in the area traversed by the blastopore during its closure, but also over a further area in its dorsal continuation. Both heads were formed approximately at the dorsal edge on both sides of the direction of the blastopore closure, that is, on both sides of the dorsal extension of the path traversed by the blastopore. The second dorsum, Rb, formed on the animal surface. It should be added that at times a whole series of small slit formations was observed on the animal surface. But, these disappeared again very quickly and cannot be apparently compared to that small animal blastopore slit, which we have often encountered in other embryos associated sometimes with formation of a second posterior end. Regarding the examinations by sectioning it may be mentioned that only the vegetal, longer dorsum of this twin formation possessed a chorda (Wittmann).

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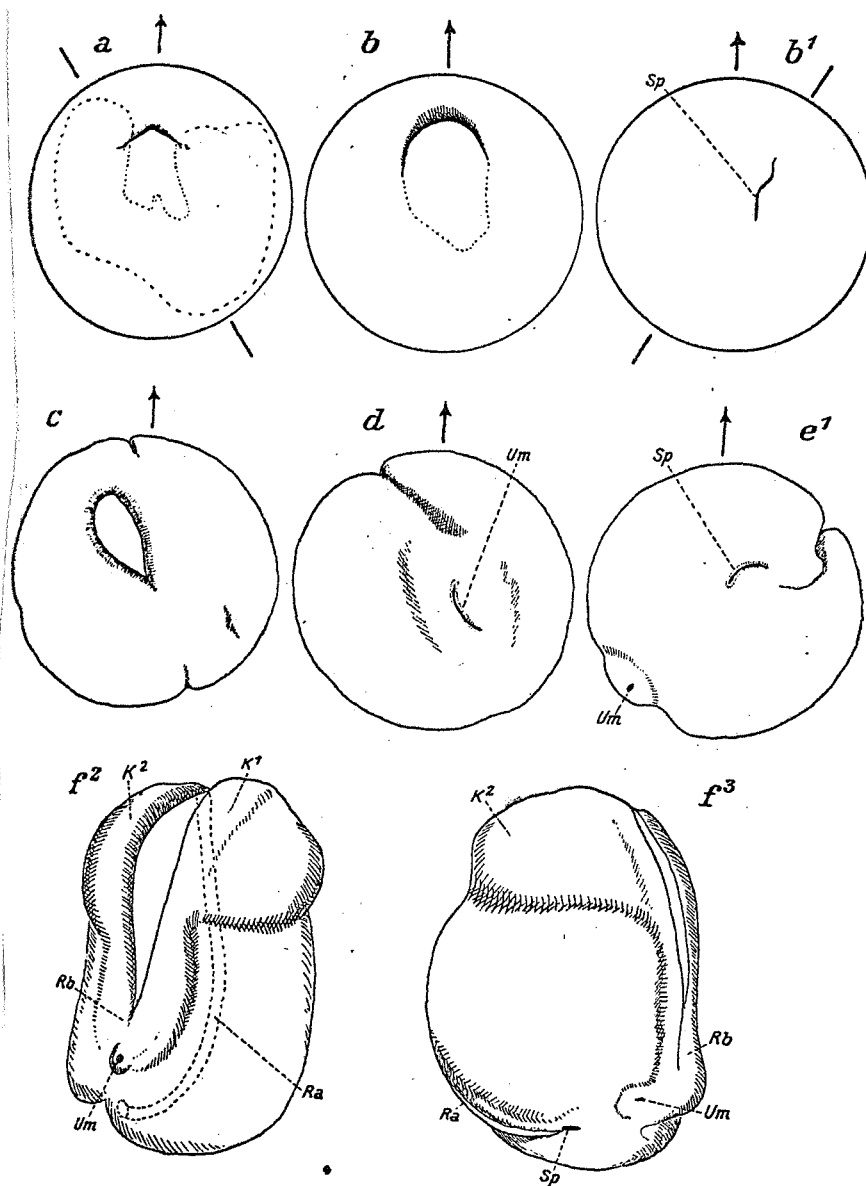
Fig. 75.

Embryo X 8. a) 1 April, 2.45 pm. b) and b¹) 7.50 pm. c) 2 April, 1.20 am. d) 7.00 am. e¹) 7.00 pm. f²) and f³) 3 April, 11.30 am; f² vegetal view, slightly right-ventral. f³ view from left, slightly more animal than vegetal. Sp = animal slit. Um = blastopore residue.

FURTHER EMBRYOS AND SUMMARY

In the case of two other embryos of this group, I 2 and X 9, gastrulation proceeded in principle in exactly the same way as in embryo X 7, which was fully discussed in the preceding section. Thus, in both cases a relatively normal yolk plug was again formed on the vegetal surface. But, it extended somewhat longitudinally in the median direction, and its descent occurred in such a way that the more dorsal part was the first to disappear, after which the ventral part disappeared, and finally, there remained at the ventral edge the final residue of the blastopore. Thus, in the case of these two embryos, as in the case of embryo X 7, we can also speak of a path of closure of the blastopore extending exactly, or almost exactly, in the median plane. The appearance of the embryonic anlagen was such as to suggest the presence of a long, narrow groove approximately in the region and direction of the median plane. We cannot state how the involutional processes proceeded for these embryos.

Two further embryos (II 8 and 9) should also be mentioned here. Observations on them stopped at such an early stage that we cannot say anything about the position of the twins relative to the original axis of the ovum. As blastulae and during the various gastrulation stages they behaved exactly as embryo II 24, the apparently normal gastrulation of which was fully presented in Fig. 5 (Part IV). Despite this normal gastrulation, however, duplicitas cruciatae typica formed. One of these, II 8, died early. The other, II 9, was fixed in the tail bud stage.

b) Apparently normal blastopore with animal slit. Description of embryo X 8.

The apparently normal gastrulation on the vegetal side of this embryo is shown in Fig. 75 a-d: An arc groove opened ventrally evolved on the vegetal surface almost exactly in the normal position and in a quite normal form. This groove became an elongated oval one, somewhat oblique to the direction of the median plane, and it enclosed a yolk plug similarly formed. The yolk plug gradually became smaller until only a slit-like blastopore remained, located in the median plane and extending in an oblique direction from left dorsal to right ventral (Fig. 75d, Um). While this slit shortened, it shifted simultaneously in its longitudinal direction, somewhat obliquely to the right at the ventral edge, to such an extent that it

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became just visible in animal view, (Fig. 75e[1], Um). Already long before this while only half the yolk plug was enclosed by a distinct inward-turning margin (Fig. 75b), a small blastopore slit also be-

came visible on the animal surface (Fig. 75b[1], Sp). Its longitudinal direction roughly corresponded with the direction of the longitudinal extension of the yolk plug, in such a way that the longest diameter of the yolk plug and the animal slit were located in approximately the same meridian, obliquely to the median plane. This animal gastrulation slit did not appreciably change its position and shape in the course of further gastrulation. When, in the stage shown in

Fig. 75e[1], the vegetal blastopore had closed, except for a very slight residue, the animal blastopore slit still existed in con-

siderable length (Fig. 75e[1], Sp). From this embryo, a typical

duplicitas cruciata was formed (Fig. 75f[2] and f[3]). Both heads formed on the vegetal surface approximately in the region, in which gastrulation started, that is in the dorsal region. One of the heads was

turned approximately toward the right (K[1]), the other toward the left

(K[2]). One of the dorsi (Rb) was located in the region, in which the vegetal blastopore had previously been located, and over the path of closure of the blastopore. The other dorsum (Ra) was located opposite, almost completely on the animal surface. It ended approximately in the animal blastopore slit. Both dorsi were almost equally well developed. In the case of this embryo, the neural grooves did not close. It died before then.

ADDITIONAL REMARKS ON TWO FURTHER EMBRYOS AND CONCLUSIONS

Of two embryos, which behaved similar to each other, one, VI 9, died soon after the embryonic anlagen became visible. On the other hand, the second embryo, VI 8, developed up to the closure of the neural tube and even for some time thereafter. Judging by its external appearance, it was finally fixed as a ventral twin (Wittmann), even though it had appeared previously to be an undoubted duplicitas cruciata. As regards the sectional examination, it may be mentioned that the cruciata character could still be clearly proven and that each dorsum contained a chorda. In the case of these two embryos, /49

the embryonic anlagen formed in a similar way to that described exhaustively for embryo X 8. They all developed as if a long groove had been present, of which the blastopore residue, remaining after descent of the vegetal yolk plug, and the animal blastopore slit were only a part.

These embryos should be considered as Schultze's cruciatae because an individual rudiment appeared on each of the two sides of the meridian running through the apparently normal blastopore and the animal blastopore slit. Thus, the two heads are primarily single, while the dorsi are secondarily so. It is out of question that it occurred vice versa, that is, that one of the individual parts was formed by formation of archenteric roof from the apparently normal blastopore and the other from the animal blastopore slit because this is contradicted by the above-mentioned orientation of the embryonic anlage. However, it is still not known how the involution processes proceeded to formation of a cruciata.

B. Origin of Modified Forms of Duplicitas Cruciata.

By typical cruciatae, which have been discussed in the preceding   /49
section (V, A), we understand cruciatae which clearly permit the recogni-
tion in the medullary-plate state of the characteristics of the Schultze
type, that is, combination of two primary individual front ends and two
secondary individual posterior ends, and in which the four parts of the
janus formation are completely, or almost completely, developed. It
has already been stated above that the fate of these typical cruciatae
may vary.

Of the typical cruciatae which were discussed in section V, A,
16 embryos died soon after the appearance of the embryonic anlagen,
that is still in the state of the typical cruciatae; 15 embryos were
fixed in the same state; 1 embryo (II 9) developed still further and
kept the character of the typical cruciata; 2 embryos (VI 6 and 8)   /50
developed even further and became a duplicitas ventralis; 2 embryos
(I 1 and IX 1) resulted in a single formation.

Thus it can be seen that a typical cruciata can be modified
by passing into another form of double formation. Since four of the
five embryos, which showed further development, changed in this way
it is practically certain that most of the prematurely dead or fixed
typical cruciatae would subsequently have undergone further changes.

In this section B, we shall discuss modified cruciatae; by
this term we understand double formations formed ontogenetically as
cruciatae of the Schultze type, but which change during the course
of their further development either into another double formation
or into a more or less single embryo.

From what we have said above, such modified cruciatae may be
formed from typical cruciatae. They may also form from Schultze
cruciatae, which from the beginning show incomplete or small parts
of the janus formation; that is, they cannot be classified as com-
pletely typical according to the definition of typical cruciatae.
Of course, no definite demarcation can be drawn between the former
and the latter, so that in section A, a number of cruciatae were
discussed which cannot be classified as completely typical.

The internal organization of the modified cruciatae and of
typical cruciatae is described by Miss Wittmann; they are evaluated
here as double- or single formations only from their outward appearance.

1. Duplicitas Posterior, Genetically a Duplicitas Cruciata With One Rudimentary Head

Already among the typical cruciatae we have observed cases in
which one of the two heads was less well developed, for example, in
embryos II 1 (Fig. 52), II 4 (Fig. 57), II 3 (Fig. 59) and V 1 (Fig. 67).
We have also tried to show in our first communication by means of the
illustrations in Figs. 5-7 (1925, pages /129-130), that posterior
duplications, which appear to be completely characteristic, can origin-
ate ontogenetically from a cruciata. This happens if one of the two
heads is only weakly laid down and then does not develop further
finally undergoing total regression. This shows that all transitions   /51
must be present between an embryo, which can be classified as a dupli-
citas posterior and a cruciata.

a) Still fairly typical forms of cruciata in the shape of dish-and-lid
embryos with rudimentary "lid-head" and a well formed "dish-head".

Description of embryo II 10.

Gastrulation occurred at two different points on the surface,
namely first at the right horn of the gray crescent in the white band
(Fig. 76a) . Groove formation in the left horn of the crescent started

only after 5-1/2 hours had elapsed (Fig. 76 b and b^1). Both groove
segments lengthened at the right or left edge of the embryo respectively
and were joined on the vegetal surface into a united groove (Fig. 76 c

and c^1). The grooves also joined up on the animal surface to give a

narrow circular groove (Fig. 76 d and d^1). The fact should be noted that
even from the outset the groove formed not precisely in the meridian, but
a little dorsally from the front, and approximately parallel to the
meridian and it as a whole then shifted quite markedly in a dorsal
direction (cf. Fig. 76 a-d). This shift in the dorsal direction was
more pronounced on the right than on the left, so that the position
of the groove changed gradually from parafrontal to oblique (Fig. 76 d

and d^1). At the time of the complete circular groove it therefore
separated a smaller half-embryo located more dorsally, from a larger
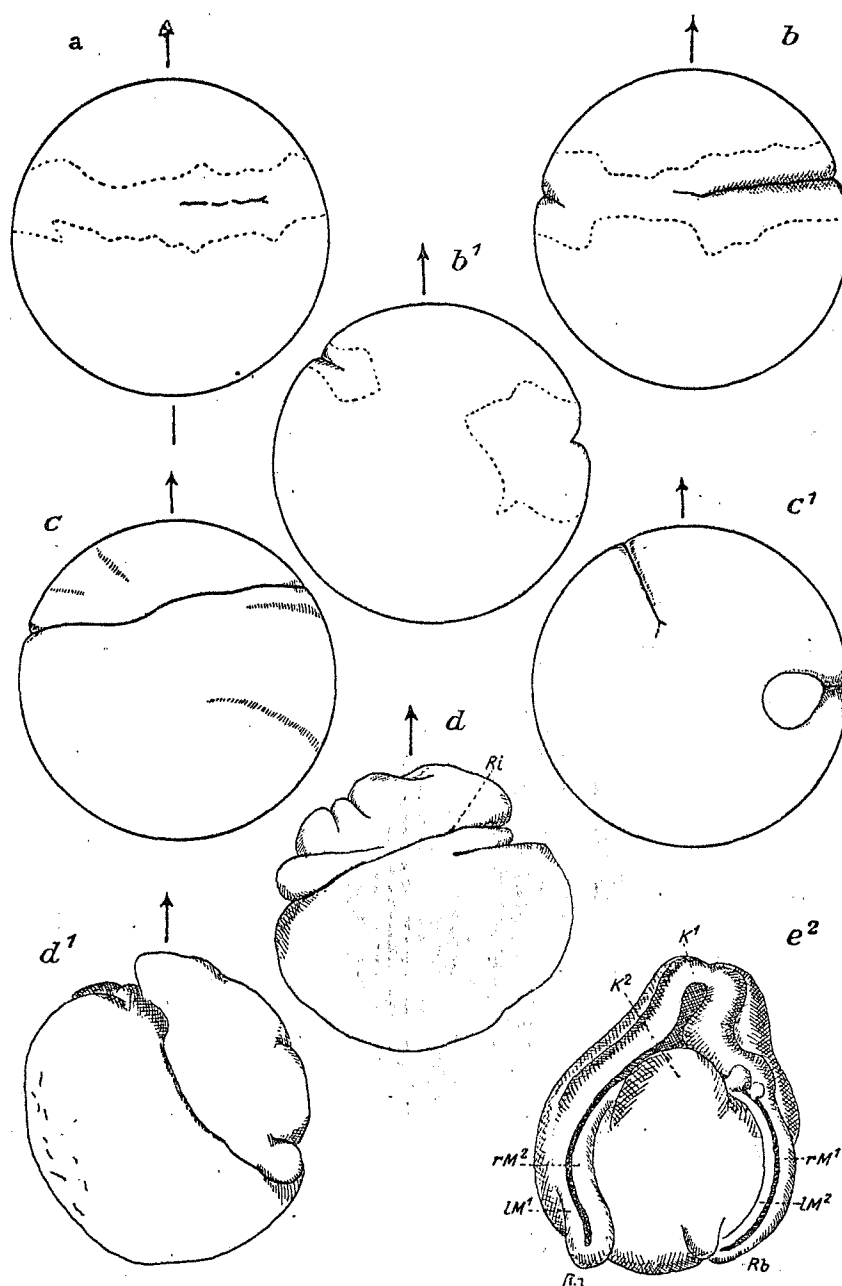
Fig. 76.

Embryo II 10. a) 12 March, 3.30 pm. b) and b^1) 8.00 pm. c) and c^1)
13 March, 5.30 am. d) and d^1) 2.45 pm. e^2) 14 March, 12.20 pm;
left dorsal view. Ri = blastopore groove.

one, located more ventrally.

In the meantime, a number of ridges and humps had formed on the smaller dorsal half-embryo. With the passage of time, they became more pronounced, especially in the region of the groove. It was not clear, therefore, how far the groove had closed at the time of the appearance of the embryonic anlagen. They were clearly formed approximately 46 hours after start of gastrulation, namely, in the form of dish-and-lid embryos (Fig. 76 e^2). The rudimentary "lid-head" (K^2) formed in this specimen on the dorsal side of the original groove, and the strongly developed "dish-head" (K^1) on its ventral side; both heads were formed at the right edge, that is approximately in the area where the groove was initially well developed, that is, the right horn of the gray crescent. This embryo still clearly showed the cruciata-character, but it could already be considered with good reason as a duplicitas posterior since the dorsal head was hardly developed and the formation gave the general impression that the two dorsi diverged obliquely from a single head (K^1) in the posterior direction. Anteriorly, however, they enclose a second rudimentary head (K^2).

Description of embryo III 1.

Figs. 77a and b show the apparently quite normal gastrulation process of the embryo. On the vegetal surface there appeared at first an arc-shaped groove ventrally opened at the dorsal edge of the bright-white complex which closed to form a round blastopore with a corresponding yolk plug at the ventral edge. From this there appeared, after descent of the yolk plug, a narrow, short slit (Fig. 77c) at the ventral edge. At the same time a bulge narrow at the surface and broad at the edges rose above the area traversed by the dorsal blastopore lip on the vegetal surface and also dorsally in the opposite direction. This bulge extended obliquely to the median plane (Fig. 77c). In this stage the embryo gave the impression of a posterior end at each end of the above-mentioned bulge (Ra and Rb). At right angles to it, in the area of the origin of the initial blastopore, and to the right and left of the bulge, there developed one head each on the vegetal surface (K^1 and K^2). In fact, the embryo continued to develop along these lines. Fig. 77 d^2 shows the embryo approximately 15 hours later in right-dorsal view. We have here a typical dish-and-lid

embryo with a large "dish-head" (K^2) and a small "lid-head" (K^1). The
first is situated more dorsally, the latter more ventrally. One dorsum
(Rb) has developed over the area traversed by the dorsal lip of the
blastopore during the closure of the blastopore. The other (Ra) lies
in the extension of the first, in the opposite direction. That is where,
judging from the picture of apparently initial normal gastrulation, the
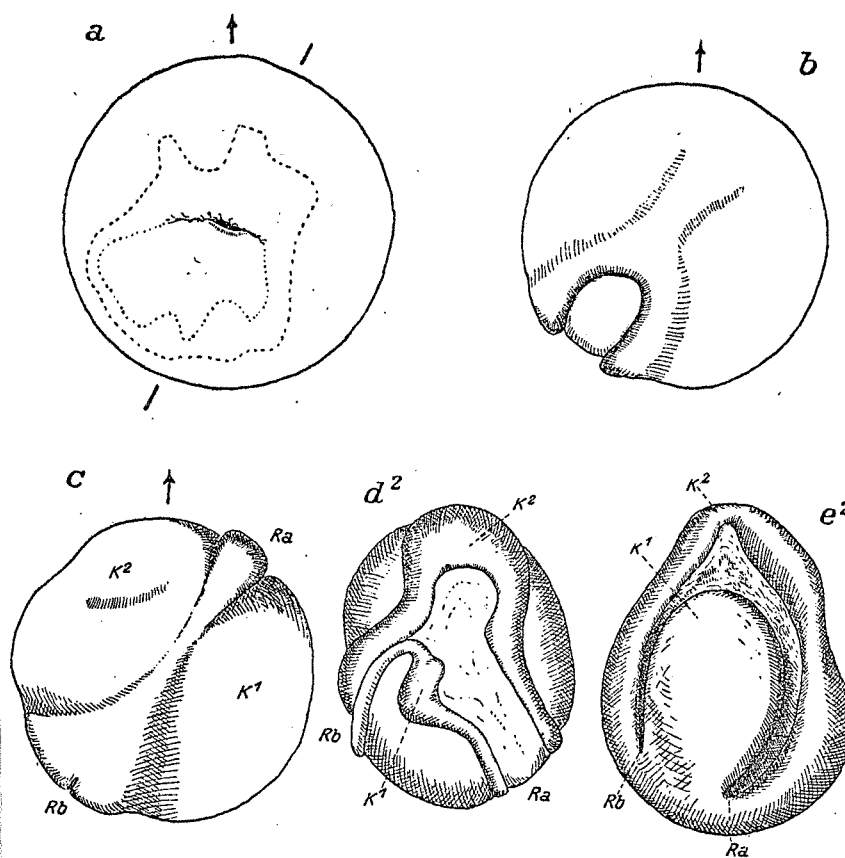
Fig. 77.

Embryo III 1. a) 12 March, 5.45 pm. b) 13 March, 4.35 am. c) 2.40 pm.
d^2) 14 March, 5.20 am; right-dorsal view. e^2) after fixation on March
14th at 12.15 pm; the same view as in d^2.

head anlage, to be expected only as a single formation, should have occurred. During further development, the "lid-head," which elevated in the stage just discussed as a distinct bulge very rapidly flattened out more and more. When the embryo was fixed after 7 hours in the stage indicated in Fig. 77 d^2, it presented the picture in Fig. 77 e^2.

Sectioning (Wittmann) showed that K^1 as such was hardly present; it existed, rather, as a simple link between the two neural folds of the more ventrally situated individual parts. It was furthermore shown that only the dorsum Rb is equipped with all the axial organs, while the dorsum Ra has no chorda. This agrees with the fact that it developed in an area not traversed by a blastopore. Even in this case, in which gastrulation occurred not with groove formation, but largely in normal conditions, there was still cruciata formation. However, it had the peculiarity that it represented a dish-and-lid embryo, the "lid-head" of which was weakly laid down from the beginning and which then took on a more and more rudimentary character so that the embryo gave the impression of a duplicitas posterior.

Additional information on two further embryos.

The gastrulation of embryo X 11 has been fully described in section IV (p. /365 and Fig. 6). At first a broad groove appeared, which later became narrow and almost completely encircled the embryo on the left in a paramedian direction. In this embryo, a larger (right) half was thus separated from the smaller (left) half. Here again, a dish-and-lid embryo with rudimentary "lid-head" was formed (Fig. 78). The total impression given by this embryo, was the same as in case II 10, except for the different position of the embryos relative to the median plane.

In the case of embryo X 12, a broad groove also formed in a similar manner as in embryo X 11. But, the narrow groove, formed from it was only short. It should be particularly pointed out that even this short groove had a paramedian position. If it had been long enough, it would have separated the larter left half-embryo from a smaller right half-embryo. The dish-and-lid embryo formed from this embryo again had a rudimentary "lid-head". One of the dorsi extended over the area of the original groove, while the other was formed opposite it on the animal surface. No gastrulation groove could be observed there. This is a behavior which we often observed in the case of typical cruciatae with short blastopore grooves.
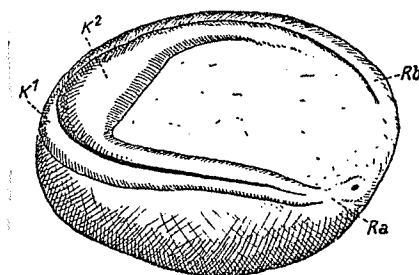
Fig. 78.

Final stage of embryo X 11, the gastrula-
tion of which is shown in Fig. 6 (Part IV);
view from left, after fixation on April 4th.

SUMMARY

Thus we see that such dish-and-lid embryos with rudimentary
"lid-head: are in terms of the anlagen without doubt cruciatae, but
can take on the character of a duplicitas posterior if one head
anlage remains rudimentary. This type of development can occur after
the most varied forms of gastrulation: from an initially narrow
groove (and even a circular one), from a broad groove (long as well as
short) and, finally, even from an apparently normal blastopore forma-
tion. In the cases of groove formation described , this groove on
the surface of the embryos was asymmetric, that is, it was positioned
paramedianally so that in a way it separated the embryo into two
halves of unequal size. The rudimentary "lid-cover" was formed in
every case on the smaller half-embryo. Thus, it appears that this
position of the groove, which appears during gastrulation, basically
determines that the head of the rudimentary embryo, which stems from
the smaller half-embryo, remains rudimentary. But, according to
the above, the same type of double formation can also occur for other
types of gastrulation. Thus, the outward course of gastrulation is not
the only determining factor for the developmental result. Fig. 76 e^2

exemplifies in diagram form this type of modified cruciata.

b) Other cruciatae with a rudimentary head from the outset.

Description of embryo IX 4.

Fig. 79a shows a blastopore groove narrow from the outset, and which even in its fullest stretch can only be described as short. We see further in the illustration how ridges appear ventral to the groove, which extends from left-ventral to right-dorsal in an oblique direction. Later on, they disappear, namely at the time when the blastopore has closed to a small slit and has shifted left ventral on the vegetal surface to the edge (Fig. 79b). In the meantime, signs of a neural groove have appeared on the vegetal surface approximately in the direction and position of the original groove. Nothing of note can be seen at this stage on the animal side. About 10 hours later, a pronounced cruciata-formation is seen in the embryo (Fig. 79 c and c^2). Approximately in the area of the start of gastrulation, right dorsal on the egg, two heads were laid down; one (K^1) was large and well developed, the other (K^2) only indicated by a slight thickening of the neural folds lM^2 and rM^2 which pass into each other there. Both dorsi are approximately developed to the same extent, Ra on the vegetal surface above the original groove, Rb in its continuation on the animal side. However, the groove itself was never visible on the animal side. During the course of further development, the neural grooves closed completely and, approximately 85 hours after the beginning of gastrulation, the large head was equipped with a sucker and gill rudiments. The small rudimentary head was barely recognizable. On the following day, the embryo was fixed as a typical duplicitas posterior (Fig. 79d^3). Outwardly, rudimentary head was no longer visible. Sectioning (Wittmann) showed that while one of the dorsi (Rb) contains axial organs, they existed only in very rudimentary form. From the rudimentary head K^2, which at the time of fixation could no longer be observed externally, a small residue could still be identified internally as a brain appendage of K^1.
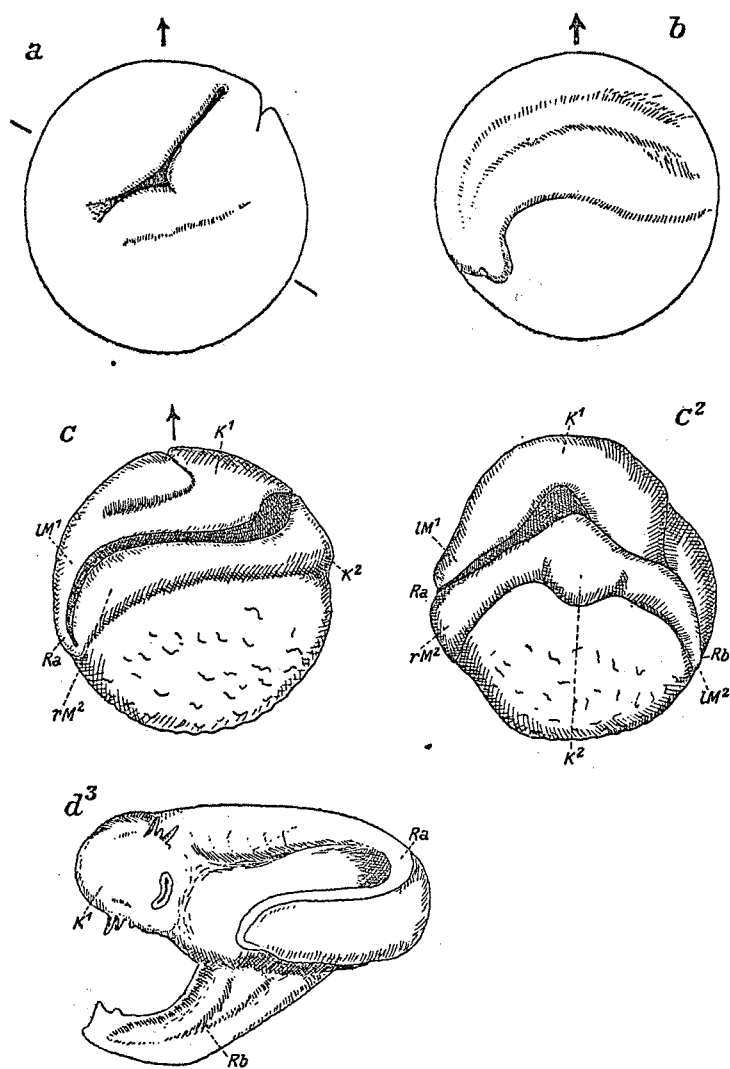
Fig. 79.

Embryo IX 4. a) 1 April, 6.50 pm. b) 3 April, 12.45 am. c) and
c^2) 10.25 am; c^2 view from right. d^3) after fixation on 5 April,
view approximately left.
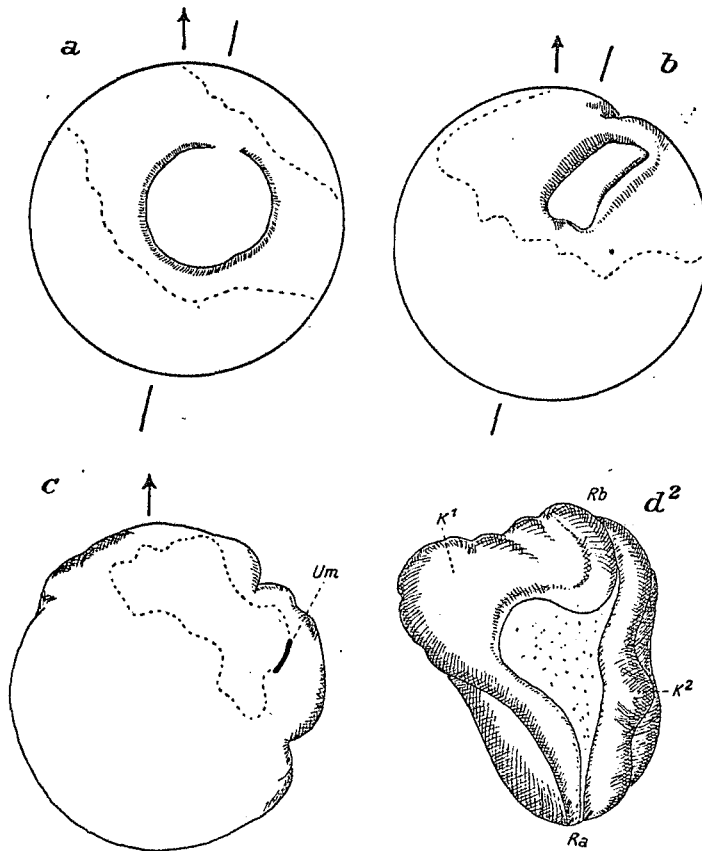
Fig. 80.

Embryo XI 5. a) 1 April, 9.15 pm.
b) 2 April, 1.30 am. c)12.30 pm.

d^2) 3 April, 10.00 am; right-
vegetal view. Um = blastopore residue.

Description of embryo XI 5.

Fig. 80a shows an apparently normal, completely round blasto-
pore on the vegetal surface. This narrowed and moved simultaneously
as a whole towards the dorsal edge (Fig. 80b). The narrowing of the

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blastopore did not proceed uniformly in all directions, but at a slower
pace in the direction right-dorsal to left-ventral. It proceeded fast-
er in the direction at right angles so that at the time when the plug
had completely descended; a short, oblique blastopore slit remained,
which soon after shortened and moved as a whole to the right edge
(Fig. 80c, Um). It then moved slightly along this right edge towards
the ventral edge. From this embryo, there developed a duplicitas
cruciata, on which one head, at the time that the embryo anlagen were
well defined, was indicated only as a small head.

Fig. 80d^2 shows the embryo in the condition when the neural
groove was still partially open. One dorsum (Ra) was formed approxi-
mately over the area traversed by the blastopore slit after descent of
the yolk plug. The two heads were formed approximately in the area
where gastrulation started and the second dorsum (Rb) in the pro-
longation of the other dorsum, but in the opposite direction. In
this stage, the embryo was fixed so that there is no way of predict-
ing how the rudimentary head anlage would have developed. But, there
is no doubt that the cruciata formation shows the tendency to change

into a duplicitas posterior with only one head (K^1).

ADDITIONAL REMARKS ON OTHER EMBRYOS.

In the case of another embryo, namely IX 5, in which gastrula-
tion proceeded apparently normally as in the case of XI 5, a duplicitas

cruciata with rudimentary head anlage also developed (Fig. 81a^2).
In the case of this embryo, one dorsal anlage (Rb) was considerably
shorter than the other (Ra) from the outset, which developed from the
blastopore residue. This embryo developed to a relatively advanced
stage and finally yielded a fully typical duplicitas posterior with
one long posterior end and a second somewhat shorter posterior end
(Fig. 81 b^2). It should be further pointed out that in the case of
this embryo, in addition to the apparently normal blastopore on the
vegetal surface, two small blastopore-like slits appeared on the
animal surface. But, it is impossible for us to determine how the
embryonic anlagen were situated in relation to these slits which
later disappeared. Also, in the case of this embryo we cannot
specify the orientation of the embryo anlagen relative to the
original axes of the ovum.

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In the case of two further embryos, V 3 and VI 11, an animal blastopore anlage also appeared in addition to an apparently normal blastopore on the vegetal surface. In both cases, one dorsum terminated in this slit. Finally, in the case of the last two embryos, IV 3 and VI 10, there appeared an animal blastopore anlage, independently of a vegetal short, narrow groove. All four embryos became cruciatae with a rudimentary head anlage. Thus, they too showed the tendency to evolve into duplicitas posterior.
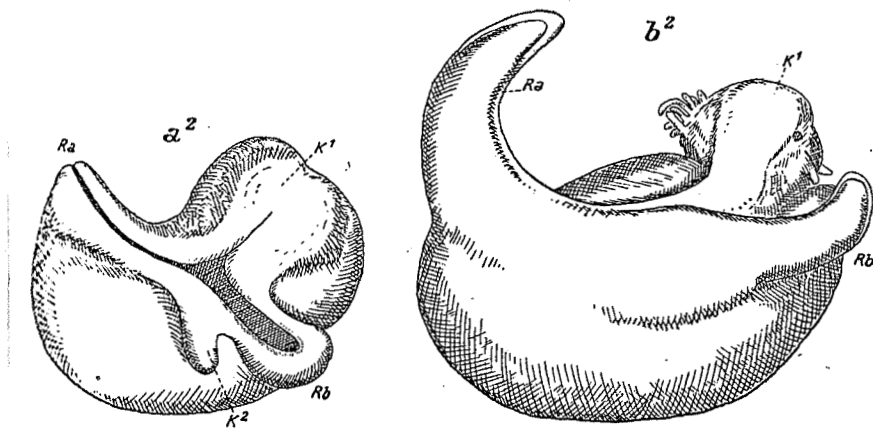
Fig. 81.

Embryo IX 5. a^2) 3 April, 10.30 am. b^2) after fixation on 5 April. Orientation relative to the axes of ovum unknown.

SUMMARY

While the embryos, which we discussed in sub-section (a) of this group, corresponded in general appearance to the dish-and-lid embryo, which was reproduced in Fig. 11 of our first communication (1925, page /132), with the characteristic that both neural grooves do not form a straight (180°) angle but a considerably smaller one

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to each other, in sub-section (b) we are dealing with forms of the same type as presented in Fig. 5a and b of our first communication (loc. cit. page /129). In these cases, both neural grooves form at the first appearance of the embryonic anlagen a completely, or almost completely, straight angle. Thus, they surround the embryo approximately meridionally and in this way more closely resemble the duplicitas cruciatae, showing regular development. But, these embryos also form one very rudimentary head, and thereby show the tendency to a double formation with one head and two dorsi, that is a duplicitas posterior. A case, in which the development advanced to a later stage than usual, finally led to a completely typical posterior duplication. This manner of development is totally independent of the outward type and manner of gastrulation Fig. 80 d^2 serve as a diagram for this type of posterior twinning duplication.

2. Duplicitas Anterior or Lateralis, Genetically a Duplicitas Cruciata with One Rudimentary Dorsum.

We saw in the preceding section that a duplicitas posterior can originate from a genetic form of cruciatae, if one head is not properly formed and finally disappears altogether. Correspondingly, anterior duplications can also evolve when, in the genetic cruciata form, the dorsum does not fully develop.

This was a conclusion already reached in our first communication from a few final stages of development (1925, Fig. 2-4) and may come about in two ways: first, one of the dorsi is only weakly laid down and does not fully develop, but it may occur because one of the dorsi, as it were, folds back over the other and also remains rudimentary. In this case it may be that forms develop, the axial organs of which are separated except for the hindmost ends; that is, they appear as lateral twins.

Even among cases of typical cruciata forms, which were described in section V, A, we have notided a few examples (embryos not described in detail: 3 (25), I 2, II 6 and 7, X 9 and XI 3) in which one posterior end was from the outset considerably shorter or weaker than the other. Furthermore, we should refer to embryo 1 (25) in which one posterior end curved inward toward both heads and appeared very short (Fig. 50). Such cases lead us to the double formations which are discussed here.

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a) Origin of a duplicitas anterior through incomplete formation
of one dorsum.

Description of embryo IV 5.

In this embryo, there developed a groove which was narrow from
the outset. It appeared on the dorsal edge in the median direction
and extended only slightly on the vegetal surface, but attained a
considerable length on the animal surface (Fig. 82 a and a^1). On
the vegetal surface, the groove did not elongate (Fig. 82 b), but
closed later on, while its animal end lengthened up to the ventral
edge (Fig. 82 b^1). But even there, the groove closed before the
appearance of the embryonic anlagen. Fig. 82 c and c^1 show the last
stage of this embryo, drawn shortly before it died.

Corresponding to the fact, that the groove did not develop on
the vegetal surface, that is, that turning inward could have occurred
there only on the dorsal edge, no medullary anlage appeared on that
side; only in the direction of the original white band could a strip
of brighter material be recognized. However, in this case, a regular
dorsum was not laid down. However, on the animal side, neural folds
formed on both sides of the original region of the groove. However,
they were not able to combine to give a single neural tube since
they were prevented from doing so by yolk material which, after the
closure of the groove, bulged outward in its region. Both neural
folds rM^2 and lM^1 touched each other posteriorly at the ventral edge.
Anteriorly, each passed into a head anlage formed at the dorsal edge,
to the right (K^1) and to the left (K^2) of the original groove. In
this case, the right and the left half-embryos did not each form a
whole embryo but only one head each and one neural fold each, right
or left respectively. The fact that these two neural folds did not
join to form a single dorsum, but instead behaved like all the four
neural folds in the Wetzel cruciata form makes no real difference to
the anterior characterior of this twin formation.

Embryo IV 7 behaved in principle in exactly the same way.

Description of embryo II 16.

In this embryo, also, there was a narrow blastopore from the
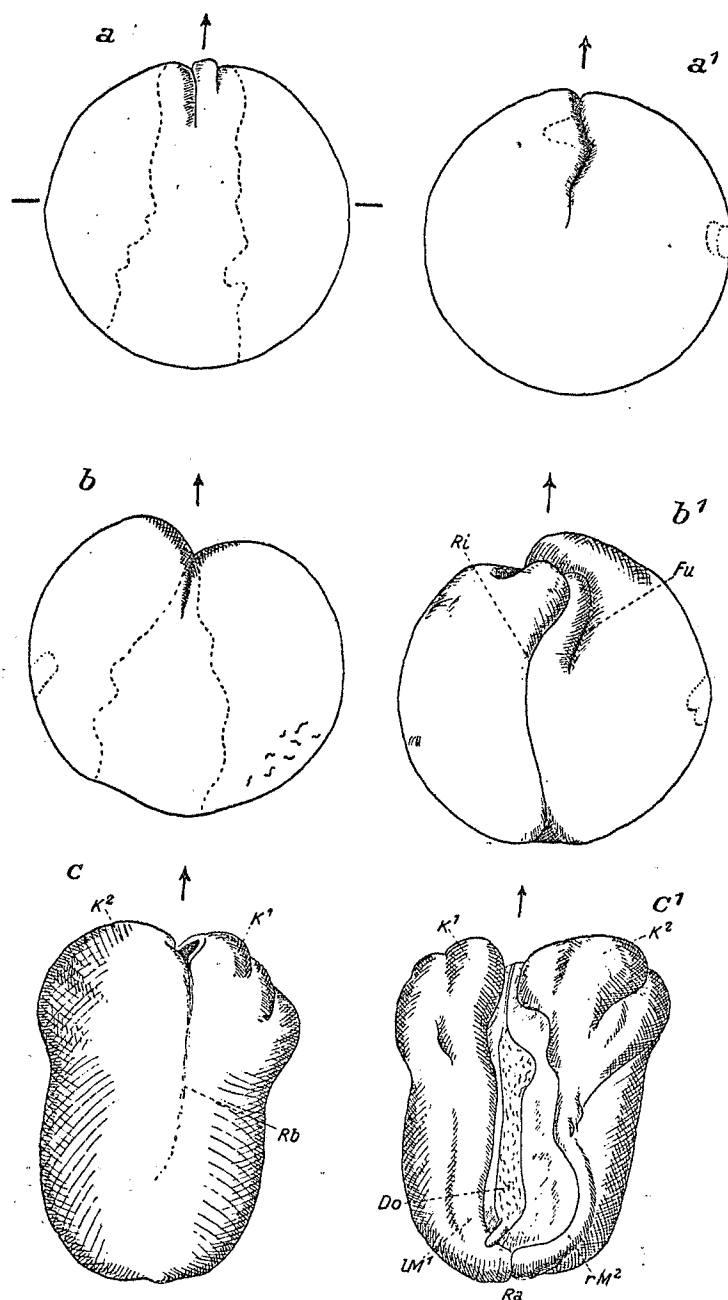
Fig. 82.

Embryo IV 5. a) and a[1]) 17 March, 12.30 am. b) and b[1]) 11.00 am.
c)and c[1]) 18 March, 11.00 am. Do = white yolk, Fu = furrow,
Ri = blastopore groove.
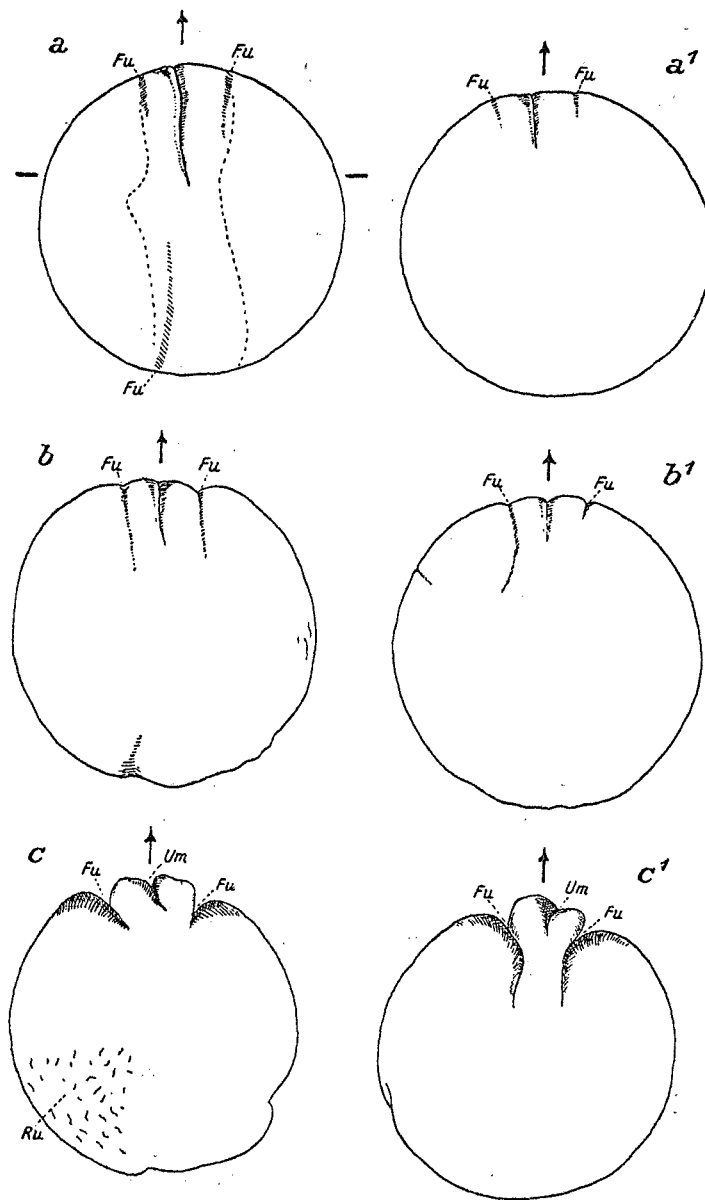
Fig. 83.

Embryo II 16. a) and a[1]) 13 March, 6.00 am. b) and b[1]) 10.00 am.
c) and c[1]) 4.00 pm. Fu = furrow, Ru = wrinkle, Um = blastopore
residue.

outset which 14 hours after the start of gastrulation extended around the dorsal edge in the median plane. On the vegetal surface it reached almost to the center, while it was considerably shorter on the

animal surface (Fig. 83 a and a^1). In this stage a furrow developed at the dorsal edge, accompanying the groove but some distance from it on either side. Another furrow appeared left ventral on the vegetal surface. At first, these furrows were not very deep or conspicuous,

but soon, the dorsal furrows deepened considerably (Fig. 83 b and b^1), while the ventral one disappeared. In its place, there appeared numerous small wrinkles (Fig. 83 c). At the same time, the groove began to close from both ends. The furrows became deep notches, and the material between them, rose at the dorsal edge quite high above the remaining surface of the embryo together with the blastopore remains.

Fig. 84 d^2 and 84 d^3 show in dorsal and ventral edge-on view, respectively, the final stage reached by this embryo. Two head anlagen

developed exactly at the dorsal edge, one (K^2) facing right, the other (K^1) facing left. On the vegetal surface, each of these head anlagen passed into a neural fold which extended around both sides of the original groove and in its continuation around the ventral edge, and which formed the neural material of the secondary vegetal dorsum Ra. A second, completely rudimentary, dorsum anlage appeared on the animal side of the embryo in its dorsal half as a narrow bulge (Rb). Since this second dorsum was hardly visible, the embryo outwardly resembled an anterior duplication. We should add that the

bulge (compare Fig. 83 c^1) which extended beyond the general embryo surface at the dorsal edge, appeared for a time as a rudimentary second dorsum. In its elongation, a whitish strip extended to the animal surface and later on transformed into the above-mentioned

narrow bulge (Rb in Fig. 84 d^3). This prominent swelling was later necrotized and then shed. In Fig. 84 d^2, a small residue (X) of it can still be recognized. Unfortunately, from our sketches, we cannot tell with certainty how this bulge (X) was connected with the rudimentary dorsum Rb.
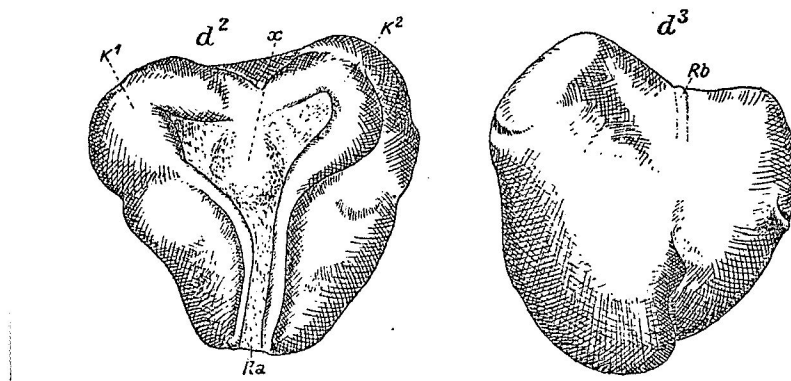
Fig. 84.

Continuation of Fig. 83. d^2) and d^3) after fixation on 14 March, 7.30 pm; d^2, dorsal, d^3, ventral edge-on view. X, site of necrosis.

SUMMARY

Thus, in these three cases, a duplicitas anterior formed from an original cruciata in the following manner. A narrow gastrulation groove was formed at the dorsal edge. It lengthened from there mainly in one direction, either only on the animal surface, or only on the vegetal surface (Fig. 85 a^2). No lengthening occurred in the other direction. Correspondingly, two heads (Fig. 85 b^2) were formed at the dorsal edge, at right angles to the groove after its closure; a long posterior end with two distinct neural folds appeared on the surface over which the groove was of considerable length, while on the other surface there was only a hint of a very rudimentary dorsum.
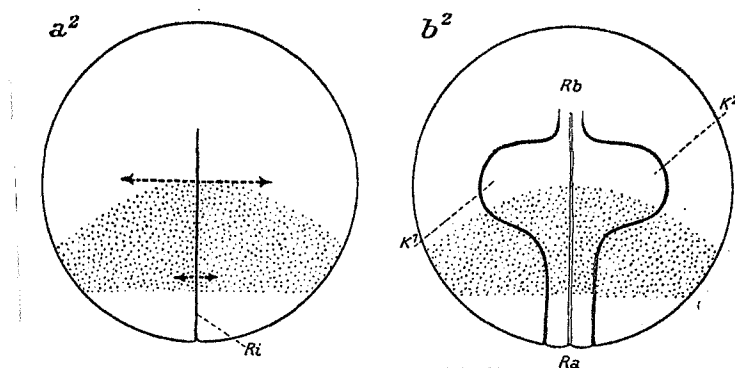
Fig. 85.

Diagram of formation of a duplicitas anterior from
a genetic cruciata form by incomplete formation of
one dorsum.
a^2) Median groove in dorsal view; b^2) Embryonic
anlagen in the stage of the closed neural groove.
Explanation as in Fig. 55.

b) Origin of a duplicitas anterior or lateralis by displacement
of the point of intersection

Description of embryo I 3.

At the time of start of gastrulation, the embryo appeared in
vegetal view exactly as embryo I 1, the gastrulation of which is
presented in detail in Fig. 8. Here also, a U-shaped blastopore
formed in the approximately normal position but soon changed to a
narrow groove, one end of which ran over at the dorsal edge to the
animal side. The other end lengthened by branching on the
vegetal surface and there embraced the yolk plug (Fig. 86 a) and
finally descended at the ventral edge (Fig. 86 b). In the mean-
time, the groove had begun to close from its dorsal end. After
complete descent of the yolk plug, there remained of the groove
only a cavity-like blastopore residue in the vicinity of the ventral
edge, and at the same time the embryonic anlagen developed (Fig. 86c).
Above the area of the original groove, a bulge now appeared which
was very broad dorsally, considerably narrower at the center of the

vegetal surface and again somewhat broader at the ventral edge.
To the right and left of this ridged closure of the groove there
were two wide bulges, which could be considered as head anlagen K^1 and
K^2. They were somewhat closer to the dorsal than to the ventral edge,
and had thus been laid down approximately to the right and left of
the point of origin of the groove. The ridge appearing on closure of
the groove must be regarded at both its ends as Ra or Rb respectively.
Accordingly, the twins had the shape of a cruciata. However, 13-1/2
hours later the embryo represented a duplicitas lateralis (Fig. 86 e).
Two neural grooves situated next to each other, extended on the vegetal
surface from the ventral to the dorsal edges, each terminating in a
head anlage. If we compare this stage with the one presented in
Fig. 86 c and further consider the intermediate stage represented in
Fig. 86 d, we come to conclusion that the point of intersection
(Fig. 86 c, +) of the cruciata, still with typical location in the
earlier stage, has shifted during further development toward the
ventral edge (Fig. 86 e, +). Thereby, the head anlagen originally
located opposite each other, came to lie next to each other. This
is explained especially by Fig. 86 d. At the same time, this also
accounts for the fact that the dorsum was drawn in the same direction
as dorsum Ra. It was pulled, as it were, in between its two neural
folds, and so showed only very weak development.

Three further embryos reached a very similar final stage in
the same way. Only the final position of the twins, in relation to
the original egg median, were very different. In the case of the
embryos IV 6 and 8, the dorsi of the embryos were situated on the
animal side with orientation the reverse of normal. In the third
twin, they were situated on the vegetal side, but transversely to
the normal direction, one dorsal and the other ventral.

Description of embryo II 17.

Gastrulation proceeded similarly to the preceeding case, but
with the difference that the groove was mainly on the animal surface.

Fig. 87 a^1 shows the groove on the animal surface approximately
14 hours after start of gastrulation. Its dorsal end, which had
lengthened a short distance on the vegetal surface, was now already
closed. The process of closure continued toward the ventral end,
while at the same time a bulge arose above the area, in which the
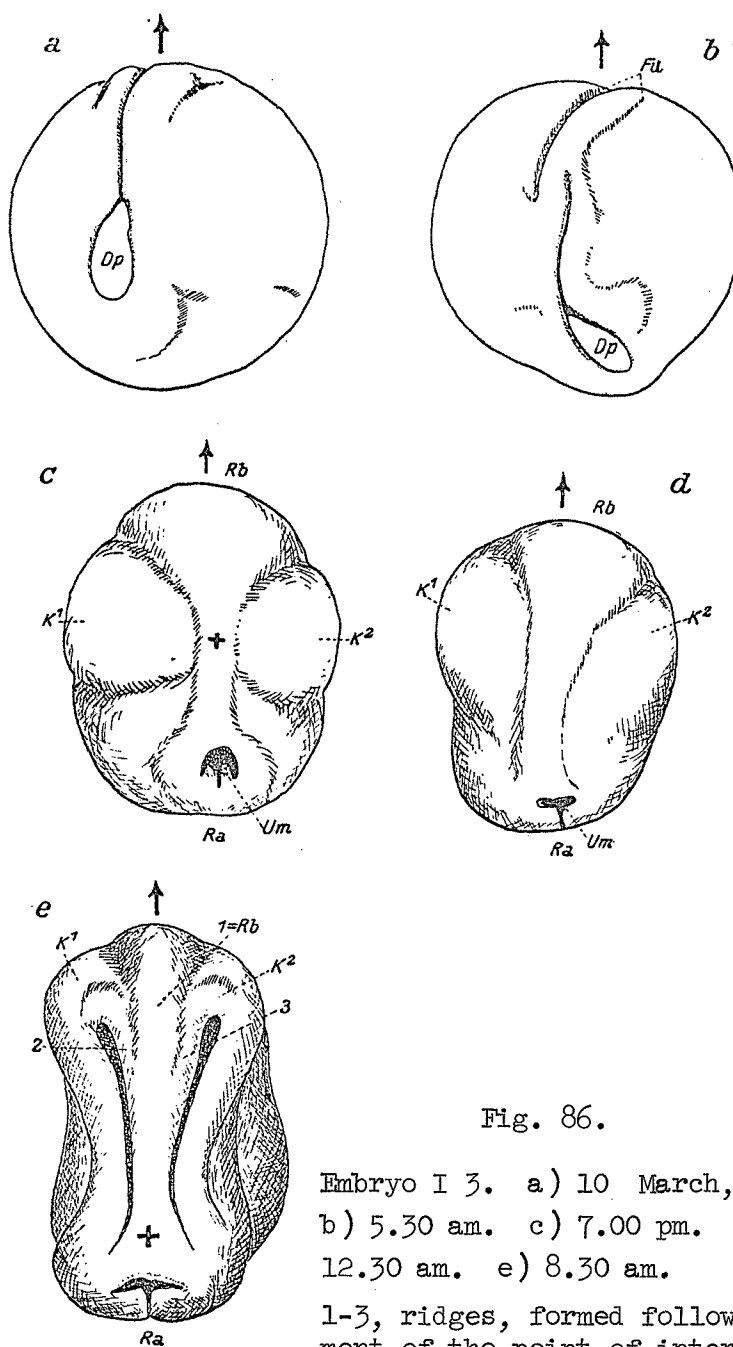
Fig. 86.

Embryo I 3. a) 10 March, 12.30 am.
b) 5.30 am. c) 7.00 pm. d) 11 March,
12.30 am. e) 8.30 am.

1-3, ridges, formed following displace-
ment of the point of intersection, +,
of the cruciata.

dp = yolk plug, Fu = furrow, Um = blastopore residue.
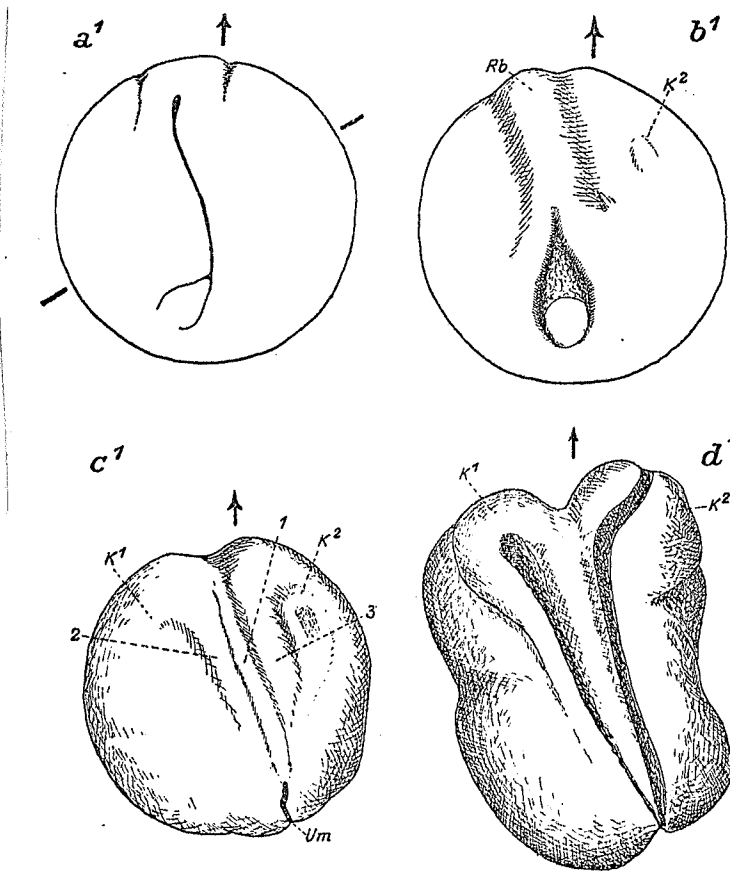
Fig. 87.

Embryo II 17. a^1) 13 March, 5.15 am. b^1) 10.40 am.

c^1) 8.40 pm. d^1) 14 March, 6.45 am. 1-3, ridges,
formed following displacement of point of intersection
of the cruciata. Um = blastopore residue.

groove was already closed (Fig. 87 b^1); the groove temporarily
widened at its end which was not yet closed, and this could also be
observed occasionally in other cases. The cruciata anlagen could

already be partially recognized as indicated by Fig. 87 b^1.

Fig. 87 c^1 represents the embryo 11 hours later. The groove had
closed, after descent of the yolk plug, except for a small slit (Um)
at the ventral edge. The ridge of closure above the groove had be-
come considerably narrower, and to its left (on the figure, to its
right) an already very clear medullary anlage (K^2) appeared. To the
right (on the figure, to the left) there was also one, but only

faint (K^1). These anlagen were now already oriented approximately
parallel to the closure ridge, but converged slightly toward the
blastopore residue. Then, an anterior duplication formed, which out-

wardly appeared typical. It is shown in Fig. 87 d^1 in animal view.
We must assume for this embryo, the same manner of formation as
described above. But, in the present case, the dorsum Rb is even less
well formed than in the other case.

A second embryo, II 18, reached the same final stage also
after the same gastrulation process. It was fixed, judging by
outward appearance, as a typical duplicitas anterior. However,
sectioning (Wittmann) showed that in reality it possessed two
chordae completely separated from one another, and which ran side by
side internally. The impression of a duplicitas anterior was only
produced by the arrangement of the externally visible medullary
material, while internally there existed rather a lateral twin.

SUMMARY

Twins appearing as duplicitas lateralis or anterior thus arise
genetically from a cruciata form in the following manner: A blasto-
pore groove generally appeared on the dorsal edge and lengthened in
one direction only, either on the animal or on the vegetal side, while
simultaneously, and thus at an early point, it began to close from the
other direction; in its place, a bulging closure ridge was formed.
Somewhere near the point of the groove's first appearance, both of
the head rudiments had formed after its closure, while at both ends
of the closure ridge, the dorsal anlagen appeared so that a cruciata
was outlined, as schematically illustrated in Fig. 88 a. This anlage
of twin formation underwent further change in the following manner:
The point of intersection (+) lengthened in the direction marked by
the solid-line arrows. The points at which the outer sides of the
neural folds curved from the head rudiments toward the dorsal anlagen
were thereby lengthened in the manner indicated by the broken-line

arrows. Consequently, three narrow folds (Figs. 88 b, 1-3) arose

between the neural folds rM^2 and lM^1 as the result of the stretching
of these points of curvature and the point of intersection (+) shift-
ed to the point indicated in Fig. 88 b. The material of the head rudi-
ments had to be stretched lengthwise in the same direction as well.
One can in fact envision all of these processes from the drawings of
both the embryos described, roughly from the sequence of figures:
Figs. 86 c, 86 d, 87 c^1 and 86 e.
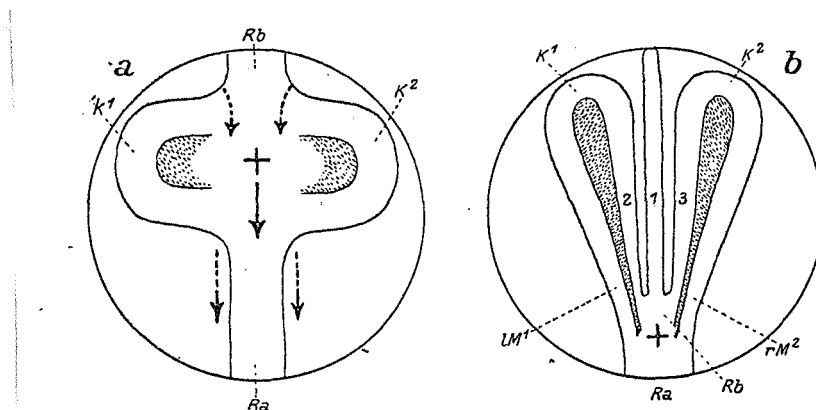
Fig. 88.

Diagram explaining how a duplicitas anterior or lateralis
originates from a cruciata due to a displacement of the
point of intersection. Explanation in the text.

3. Duplicitas Ventralis, Genetically a Duplicitas Cruciata.
a) Secondary Ventral Twins

Description of the later development of embryo VI 6.

The earlier development of this embryo, until the appearance
of the embryonic anlagen, has been described in detail above (p. /35

as well as Figs. 68 and 69). At the stage represented in Figs. 68 c^2

and c^3, a very typical duplicitas cruciata was present: Both heads
K^1 and K^2 had arisen on either side of the groove and were separated
by only a very narrow furrow. Cross-wise to them, in the direction
of the blastopore groove, one dorsum each had formed on the vegetal
and animal surfaces. The primary heads are very short in relation
to the long secondary dorsi, which have already begun to elongate in-
to tails at the time of the stage described.

The embryo was able to develop much more still and thus be-
came a modified cruciata; Fräulein Wittmann will bring an illustra-
tion of this final stage. The double formation now consists of two
embryos, which are joined ventrally and possess in common the fore-
most head segment; at this stage both the tails have grown long.   /71
This duplicitas ventralis has arisen from the cruciata by a fusion
of the anterior portions of the head rudiments, which were origin-
ally separated and laid down cross-wise to the larger posterior
body segments, so that now only one outwardly undivided anterior end
is present for both embryonic anlagen and, as a consequence, the
cruciata character is no longer recognizable. Fräulein Wittmann will
demonstrate however, that it has not been completely lost in the in-
ternal organization.

These ventral twins are to be designated as secondary because
their dorsi and tails are secondary-single formations, like those
of the Schultze cruciata in general. In accordance with the external
development, the transition between the two originally separated
primary-heads and the two secondary dorsi is far toward the anterior,
that is, two very short primary anterior head rudiments form a
cross with the two relatively very long posterior secondary segments.
As Fräulein Wittmann will demonstrate, this is confirmed in the in-
ternal organization later on.

Another embryo (VI 8), behaved, with respect to all essential
traits, in the same manner and will therefore not be described in
greater detail.

SUMMARY

A duplicitas cruciata can subsequently become ventral twins
by fusion of the two primary head rudiments into one seemingly single
head. There then remain only the secondary posterior body segments

(trunks and tails) which proceed from them. These secondary segments lie on opposite sides of the embryo and are therefore joined ventrally. Since the double formation thus essentially consists of the secondary segments, we are dealing here with secondary ventral twins. In such cases, the posterior ends are completely separated while the heads are fused.
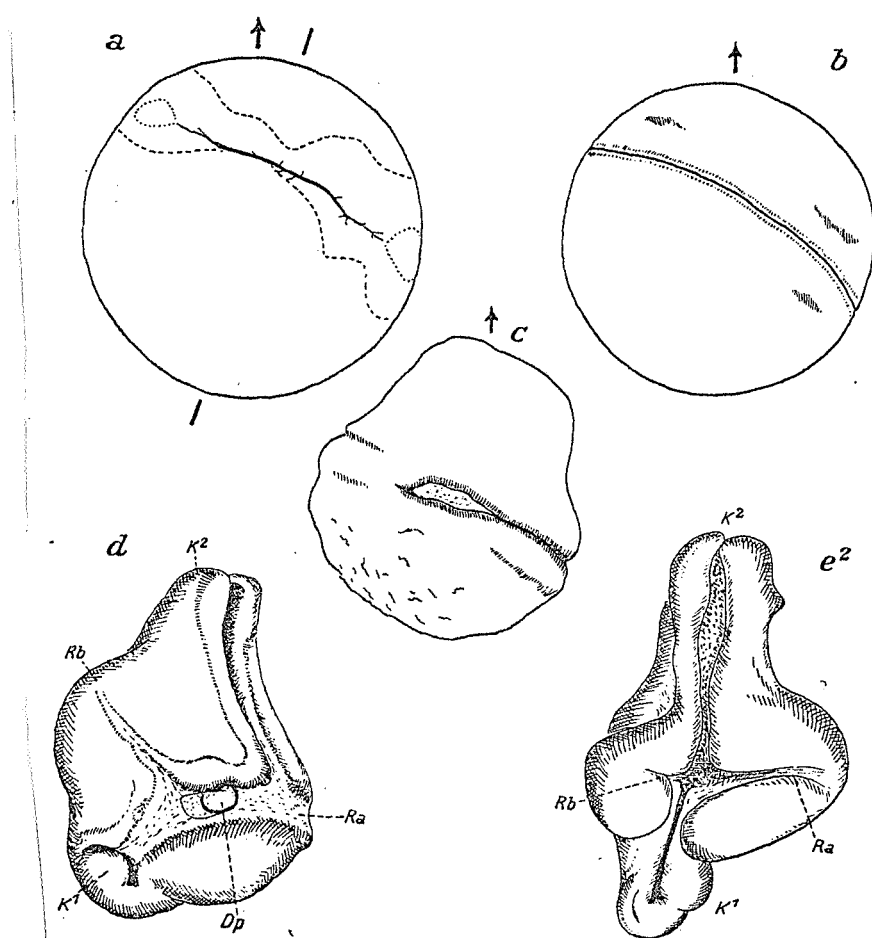
Fig. 89.

Embryo II 11   a) 12 March, 2.00 pm.   b) 13 March, 4.45 am. c) 4.30 pm.   d) 14 March, 6.00 am. e^2) 3.45 pm. right-vegetal.

b) Primary Ventral Twins

α) Definitely originating from cruciatae

<u>Description of embryo II 11.</u>

In this embryo, a narrow, long groove arose at the very be-
ginning. Fig. 89 a shows this groove at its inception, Fig. 89 b
shows it at its longest extension on the vegetal surface. The ob-
liquely situated groove overlapped a little bit on the right side
onto the animal surface, at this stage. The closure of the groove
proceeded initially from its left end (Fig. 89 c). Simultaneously,
the groove became somewhat broader on the vegetal surface since the
white yolk from the interior reappeared. Then the closure of the
groove set in also from its right end until only a round opening, at
the stage represented in Fig. 89 d, remained, that had formed on
the vegetal surface from the above mentioned broadening of the
groove. In the interior of this opening, deeply situated white
yolk (Dp) appeared. The embryonic anlagen had already appeared at

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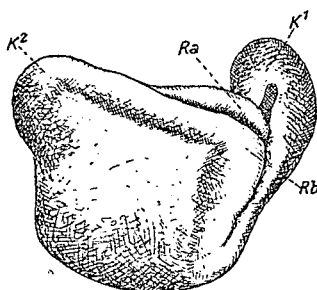
Fig. 90.

Embryo 2 (25), after fixation.

this stage, a neural groove extended from the blastopore residue

in a dorsal direction in order to terminate in a head rudiment (K^2)

near the dorsal edge, a second such groove proceeded in the opposite direction, in order to terminate in the same manner, but at the

ventral edge, in another head rudiment (K^1). Both these embryonic
anlagen of primary single origin had thus arisen ventrally and dorsally with respect to the original groove, perpendicular to its course. Along the course of the blastopore groove itself and corresponding to its position, faint bulge formations could also be recognized which are to be designated as the secondary dorsi Ra and Rb. The long primary anterior ends evolve extensively in subsequent development while the secondary dorsal anlagen undergo no further differentiation. Consequently, the duplicitas cruciata ultimately gave impression of

ventral twins (Fig. 89 e^2): Two embryos have fused ventrally,; their heads are completely separated and removed at some distance from each other; their posterior ends overlap at the point where the remnants of both dorsi, positioned cross-wise to the embryos, are still recognizable. In general, the final stage of this embryo is in approximate agreement with the double formation that is presented in our first report (1925) in Fig. 8.

Sectional examination (Wittmann) has confirmed the interpretation based on external findings as correct. There are, in fact, two complete individuals that are interconnected on their ventral sides. From the point at which the posterior ends of their axial organs are in contact, they run perpendicular to these and thus, perpendicular to the median planes of the twins as determined by the epidermal thickenings on both sides, which are to be considered as the rudimentary neural anlagen of the posterior ends. Thus, one can also recognize the cruciata character from sectional picture.

Short description of embryo 2 (25).

Here, too, a duplicitas cruciata with very short secondary dorsi that completely retrogressed later, had formed while the long primary anterior ends dominated. The embryo consequently and ultimately gave the impression of being a duplicitas ventralis (Fig. 90) in this case, too. However, it displayed the peculiarity that the one

twin (K^1) was strongly curved inward in a concave fashion, because of which the character of a duplicitas ventralis was somewhat impaired -- Embryo V 4 behaved in a similar fashion.
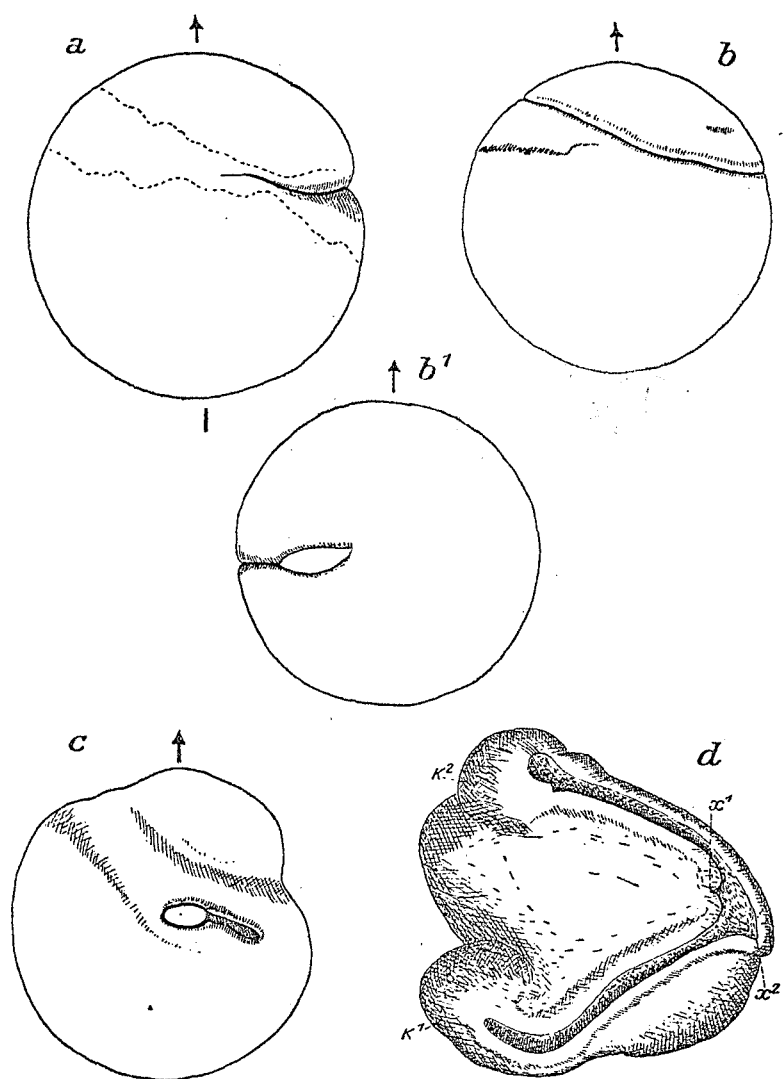
Fig. 91.

Embryo IV 4 a) 16 March, 4.00 pm. b and b^1) 17 March,
1.30 am. c) 11.30 am. d) 18 March, 1.00 pm.

x^1 and x^2 are rudimentary neural grooves of the
secondary posterior ends.

SUMMARY

A duplicitas cruciata can subsequently become a duplicitas /74
ventralis, if both the secondary posterior body segments retrogress.
Then, essentially only the two primary anterior ends remain and they
lie on opposite sides of the embryo and are thus joined ventrally.
Since the double formation then consists almost exclusively of the
two primary segments, we are dealing here with primary ventral twins.
In such a case, the heads are fully separated while the posterior
ends overlap.

β) Probably originating from cruciatae

Among the non-typical cruciatae we find relatively many in
which both trunks, that is, the secondary posterior segments, were
very weakly laid down from the beginning. Such a case exists in
embryo II 11 (Fig. 89). From this, one cannot doubt that in ex-
tremely atypical cruciatae, a differentiation of the neural folds,
which should produce the secondary dorsi, simply does not proceed
any further. If that occurs, in the Schultze's inversion experiment,
double formations can arise which develop from the outset as ventral
twins -- and indeed, as primary ventral twins.

Description of embryo IV 4.

In principle, gastrulation proceeded just as in the case of
embryo II 11 (cf. Fig. 89), in particular a long narrow blastopore
groove was formed in like manner. This did not first appear in
the middle of the vegetal surface but in the right horn of the gray
crescent (Fig. 19a). At this stage, it overlapped onto the animal
surface a little. Subsequently, a long groove arose from this, by
lengthening itself at either end (Figs. 91 b and b¹). Exactly as
in embryo II 11, the closure of the groove in this case began from
both ends (Fig. 91 c). A final residue undergoes closure in the
vicinity of the right edge. Fig. 91 d shows the twin formation which
developed from this embryo, from the vegetal view. From the region
of the blastopore residue to the right edge, one entire embryo
extends dorsally and ventrally, perpendicular to the original groove.
Both terminate at the left edge, dorsally and ventrally respectively,
in clearly defined heads. They are still at the open neural groove
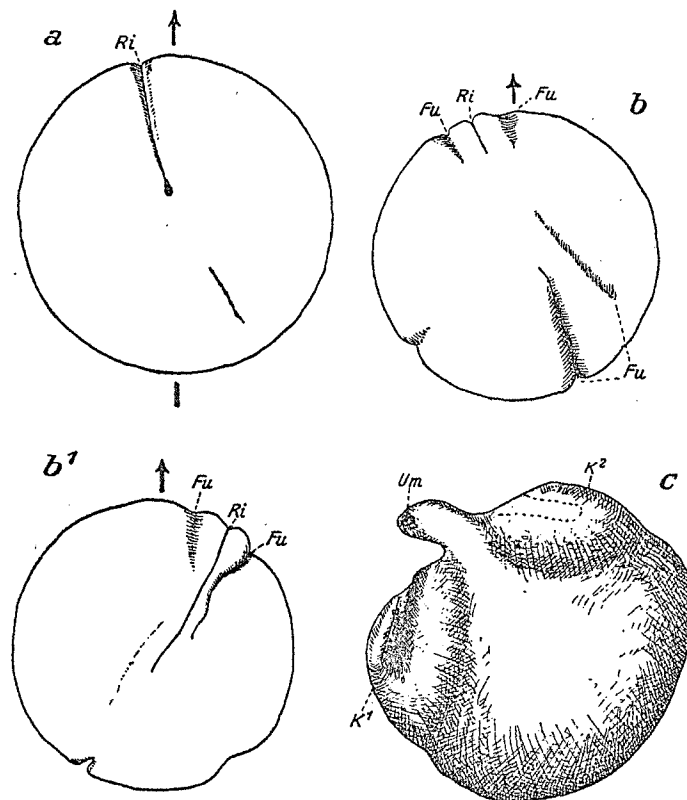
Fig. 92

Embryo VI 15. a) 24 March, 7:30 AM,

b) and b[1]) 12:20 PM, c) 25 March,
7:00 PM. Fu-furrow, Ri-blastopore
groove, Um-groove residue.
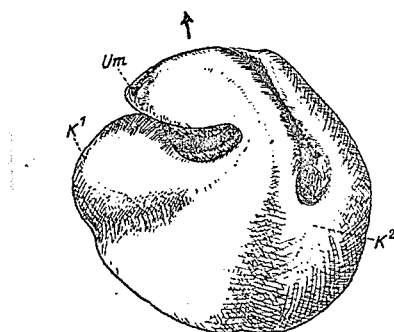
Fig. 93

Embryo VI 12. Final stage prior
to death. Um-blastopore residue.

stage. Somewhat in the vicinity of the original groove, they are
attached to each other ventrally. A very slight hint of the
cruciata character can be recognized at the posterior ends of the
neural grooves, for there, where both neural grooves join, two in-
dentations (x^1, x^2) are found perpendicular to the grooves' line of
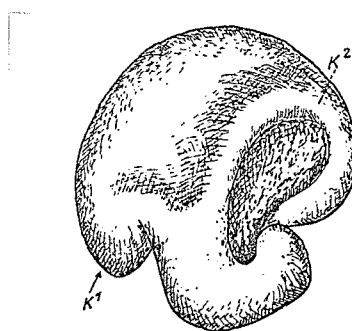
Fig. 94

Embryo VI 14. Final stage prior
to death.

orientation and these indentations are to be considered as complete-

ly rudimentary, secondary medullary anlagen. For the one (x^1), it is entirely certain that, in accordance with an earlier stage which is not illustrated here, in its place, a very short secondary neural

groove had been present. For the other (x^2), we cannot draw this conclusion from our sketches since the embryo was only depicted from the vegetal side and this indentation must be situated on the edge. But even without this indication, one must consider these ventral twins to belong genetically to the cruciata forms: Their develop-ment is, in principle, the same as that of the typical duplicitas cruciata XI 12, which has been thoroughly described in Part IV (Fig. 13) and above (Fig. 56). The only difference lies in the fact that in the typical duplicitas cruciata, from the point where the anterior ends of primary origin meet each other, two weakly developed posterior ends extend cross-wise to them whereas in this embryo, this is not the case. The posterior ends are only rudimentarily laid down and later disappear. In addition, the previously described embryo II 11 (Fig. 89) is a splendid example of a transition form between a typical duplicitas cruciata and equally typical ventral twins.

/76

/77

Description of embryo VI 15

The duplicitas ventralis in the stage of the open neural groove is reproduced in Fig. 92 c and very probably is seen from the vege-tal side. To the left, at the dorsal edge of the embryo, a high plug is upraised, on the tip of which (on the opposite side) the blasto-pore residue (Um) lies. From this spot, two medullary plates extend,

one to the left and toward the facing vegetal side (K^1), the other

to the right and growing toward the opposite animal side (K^2). The development of the embryo is briefly summarized as follows: In Fig. 92 a, a groove is seen running obliquely from the middle of the vege-tal surface to the left and dorsally. It closed toward the dorsal edge (Fig. 92 b) while, at the same time, it extended itself around

this edge and onto the animal surface (Fig. 92 b^1). At this stage, several furrows appeared, in particular, two on the dorsal edge on either side of the groove and these had the effect of causing the area delimited by them to protrude in a somewhat plug-like fashion.

This area certainly corresponded therefore, to the plug of the stage
represented in Fig. 92 c. The interpretation of this embryo may be
designated as certain: On both sides of the groove-shaped blasto-
pore anlage and perpendicular to this groove, a primary anterior end
had developed. The groove thus ran cross-wise to both these anlagen;
in the original progression of this groove, for some unknown reason
a secondary dorsum arose neither on the vegetal nor on the animal
side. Both the head rudiments shown in Fig. 92 c developed still
further and became sucker rudiments.

Additional information on other embryos

While in embryo VI 15, and also in embryo VI 16 which is not
described, both embryonic anlagen lie, on the whole, so that they
encircle the egg with their outward-curved dorsal sides; in other
ventral twins of this type, one finds that only one embryonic anlage
is curved outward in this manner, the other, in contrast, having an
inward-curved dorsum. Embryo VI 12 offers an example of this kind
(Fig. 93), as do embryos II 14 and VI 13. Moreover, as regards this
curvature of one embryonic anlage, both of the embryos shown in
Figs. 8 and 9 in our first report (Schleip and Penners, 1925) resemble
the above embryos. In addition, this concave curving may be present
in both embryonic anlagen so that both partners appear concavely
pressed against one another. When viewed in profile, a formation   /78
of this kind gives the impression of a mushroom or an umbrella, the
handle of this "umbrella" being hook-shaped in the case of embryo
VI 14.

SUMMARY

In the Schultze inversion experiment, double formations occur
which, at no stage of their development possess a truly cruciata
character but immediately appear as ventral twins. But these too can
be conceived of as genetic cruciatae with a very high degree of
probability, even though their secondary posterior ends retrogress.
There are two reasons to support this conception. First, gastrula-
tion occurs in such embryos exactly as in others which develop into
cruciatae. Second, in one of the embryos described (Fig. 91), some
indications, though very inconspicuous, of neural grooves correspond-
ing to the two secondary posterior ends could be determined.
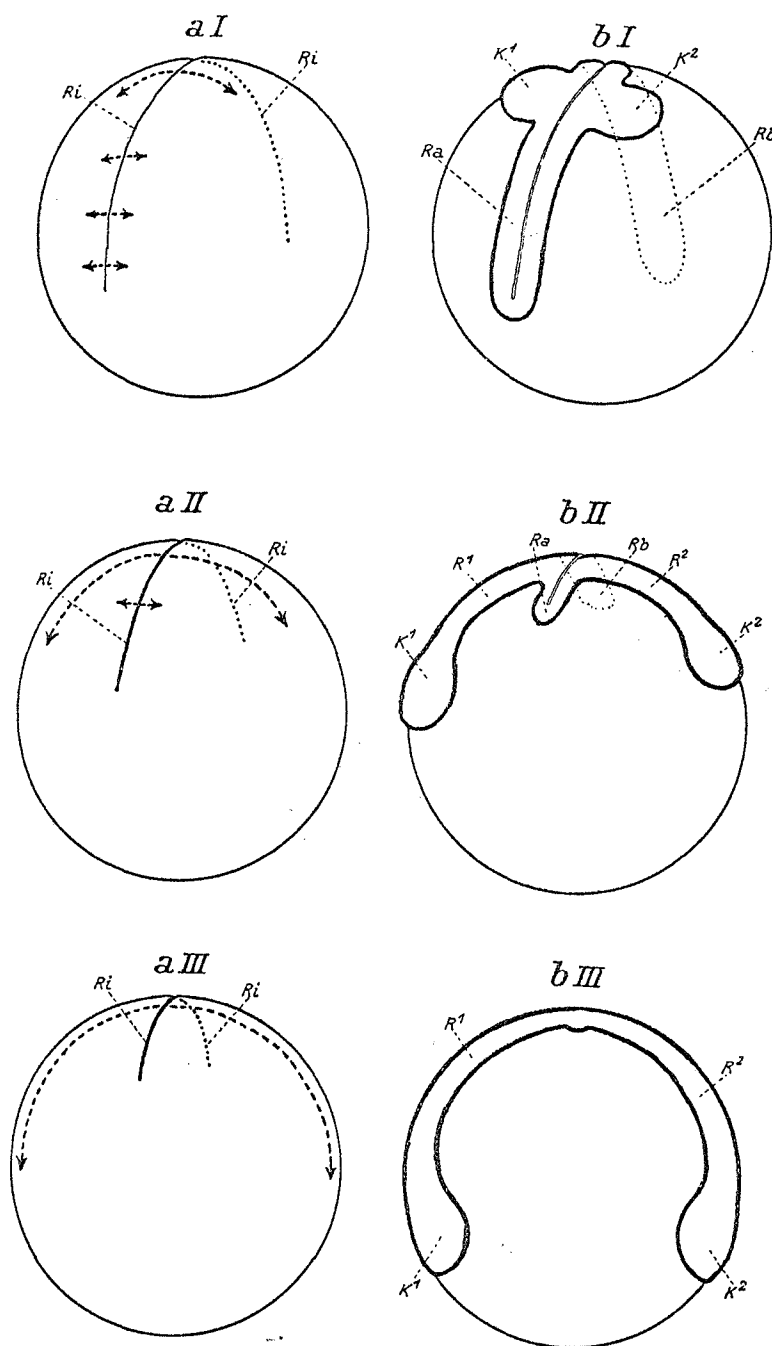
Fig. 95

Schematic of the origin of ventral twins which belong genetically to the cruciata form. Explanation in the text.

c) Schematic summary of the conclusions on the origin of ventral twins

With the aid of the schematic Fig. 95, we have summarized the previously established derivation of ventral twins from cruciatae; here we proceed from gastrulation in the form of a long narrow blastopore groove; on both sides of the middle of this groove a primary anterior end is laid down over the invaginated archenteric roof (long arrow; the involution of the remaining region of the blastopore groove, which leads to the formation of the neural folds of the secondary posterior ends, is denoted by short arrows).

1. Both archenteric roofs on either side of the middle of the blastopore groove are very short. (Fig. 95 a I) and therefore the corresponding primary anterior ends also are very short, while the secondary posterior ends oriented cross-wise to them are very long (Fig. 95 b I). When both anterior ends fuse into a single structure, secondary ventral twins with separated posterior ends and a common head evolve. /80

2. Both archenteric roofs on either side of the middle of the blastopore furrow are very long (Fig. 95 a II), therefore, the corresponding primary anterior ends also are very long, while the secondary posterior ends oriented cross-wise to them are very short (Fig. 95 b II). Primary ventral twins with separated anterior ends and scarcely recognizable secondary posterior ends thus evolve.

3. If there is no involution at all of the material in the region of the ends of the blastopore groove, then the secondary posterior ends (the two pairs of neural folds, which fuse into the secondary posterior ends) are not laid down at all (Fig. 95 a III) and only very long archenteric roofs form on both sides of the middle of the groove. Thus, only the two primary anterior ends arise from the beginning so that primary ventral twins with separated heads and a common posterior end result (Fig. 95 b III).

All the ventral twins described gastrulated with the formation of a narrow, more or less long blastopore groove. But since cruciatae also are formed by other forms of gastrulation, presumably, ventral twins will be able to originate by those other forms as well.

4. Single formation, genetically a duplicitas cruciata

Among the most remarkable phenomena in the Schultze experiment is the appearance of single formations which, according to the anlage, are to be considered cruciatae; they can arise in various ways.

a) Duplicitas cruciata with a rudimentary head and posterior end

In our first report (Schleip and Penners 1925), in Fig. 10 a, a duplicitas cruciata is shown which exhibits the peculiarity of having a head and a posterior end that have been rudimentarily laid down from the beginning. During the further development of this formation, the rudimentary nature of the parts named became still more apparent (l. c. Fig. 10 b). We have already, among the above-mentioned cases of duplicitas cruciatae described as typical, become acquainted with one example (X 7, Fig. 74 f^2) in which one posterior end as well as one head are much more poorly developed than both the other components of the cruciata. This case may be considered as a transition to the formations within the group below.

Description of embryo III 2

In this embryo a short, somewhat oblique groove appeared at first in the middle of the gray crescent, within an obliquely running white band (Fig. 96a). Since it branched on the vegetal surface around a complex of bright-white material, it subsequently broadened. This white material descended on the spot and there remained on the vegetal surface a slit lying in between the evolving head rudiments (Fig. 96 c). The duplicitas cruciata which arose from this embryo is not to be designated as typical. For it showed from the beginning,

as the embryonic anlagen came clearly into view (Fig. 96 a^2) the

peculiarity, that one head (K^2) and one posterior end (Rb) were only very weakly laid down; to the right and left of the original site of the groove, where the above-mentioned slit remained, the two heads were laid down, somewhat displaced to one another, the left one large and the right one small. An explicit, long neural groove appeared, running ventrally in the direction of the groove. As it lengthened in the dorsal direction, a second very short neural groove appeared. At this stage, the embryo was fixed. By then it gave the impression of a single formation to some degree, this being most

clearly expressed by the fact that the primary single anterior end K^1 and the secondary single posterior end Ra lay in approximately the same median plane and both the other components of the cruciata formation now appeared only as appendages on the right of the embryo.
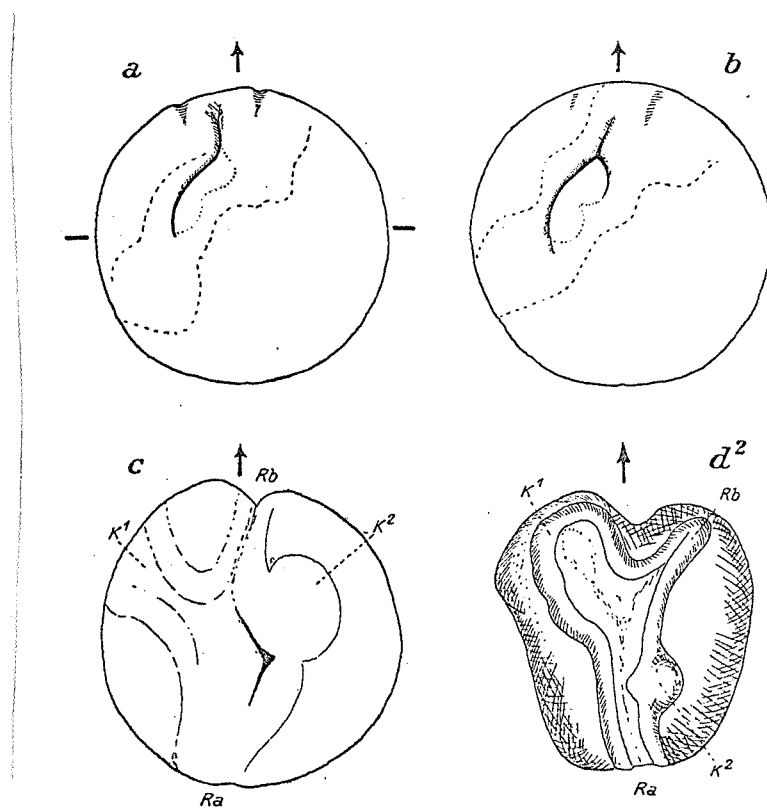
Fig. 96

Embryo III 2. a) 12 March, 2:30 PM, b) 13 March, 5:30 AM, c) 2:15 PM, d^2) 14 March, 12:15 PM; dorsal view.

Description of embryo X 6

In this embryo, gastrulation took place with the formation of
an apparently normal blastopore, since a short, dorsally convex
groove, first developed in a horseshoe shape at exactly the normal
spot. This horseshoe-shaped furrow closed, its ends enclosing a
bright-white material like a yolk plug and then sinking. Finally

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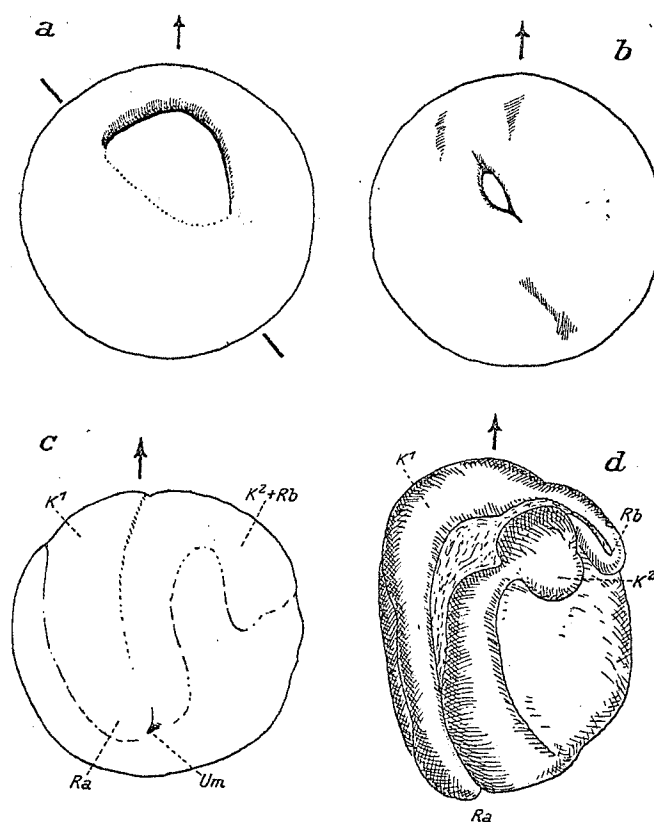
Fig. 97

Embryo X 6. a) 1 April, 7:40 PM,
b) 2 April, 1:15 AM, c) 7:00 PM,
d) 3 April, 12:00 noon. Um-blasto-
pore residue.

only a small slit remained from the blastopore; this slit moved in the median plane toward the ventral edge until the time when the embryonic anlagen appeared (Fig. 97 c, Um). At this stage, the embryo appeared in the vegetal view as a young neurula, which ex-

hibited a right-dorsal irregularity (K^2 + Rb). Somewhat later, the embryo presented the same picture in principle as that previously described (Fig. 97 d): duplicitas cruciata at the stage of a still

partially open medullary plate, in which a head (K^2) and a posterior end (Rb) have been only very poorly laid down. Soon afterwards the embryo was lost.

Description of embryo II 19.

Apart from the frontal location of the groove, gastrulation in this embryo took place exactly as in the case of the first embryo to be described in this section, III 2 (Fig. 98 a). The duplicitas cruciata which developed from this embryo, is shown in Fig. 98 b in the young neurula stage. At both sides of the place at which the blastopore groove had first appeared, head rudiments had formed,

dorsally, a large one (K^1) and ventrally, a small one (K^2), which only appeared as a tiny button. To the left, above the groove region, an explicit long and broad medullary plate had arisen (Ra), to the right, a narrow and short one (Rb). The large head rudiment and the large dorsum rudiment are almost at right angles to each other, which is typical of a cruciata formation. During further development, these two components of the duplicitas cruciata extended in one and the same direction (Fig. 98 c). Simultaneously, the rudimentary posterior end bent itself in such a manner that it came to rest on the right side (relative to this single formation) of the main dorsum (Ra). The rudimentary head could still be recognized as a very tiny button. The embryo still remained alive for a

fairly long period of time and Fig. 98 d^2 presents it in side view two days later. It is a single formation with a completely closed medullary plate. To the right, in front, on the head, a small

bulge is visible. It is the residue of dorsum Rb and head K^2.

Additional information on other embryos

The embryo IX 7 developed still further than the one just
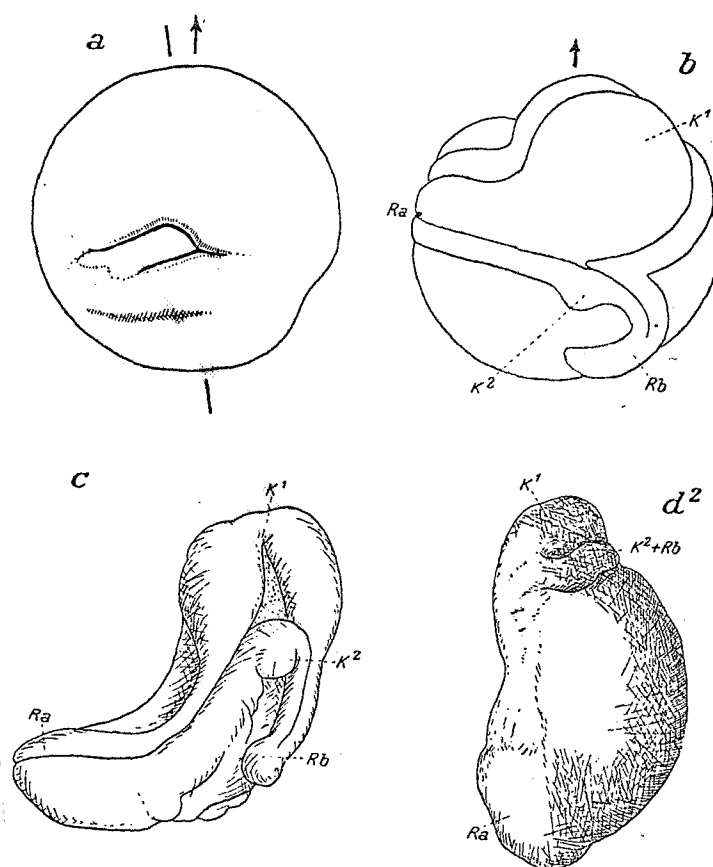
Fig. 98

Embryo II 19. a) 13 March, 12:00 noon,
b) 14 March, 7:00 PM, c) 15 March, 9:00 AM,

d^2) after fixation on 17 March, 5:00 AM;
ventral view.

described. Fig. 99 a^2 shows it at the stage of the closed medul-
lary plate. Again we see, on the whole, a single embryo which, left
of the anterior end, displays two small bulges representing the two
rudimentary components of the cruciata formation; Fig. 99 b^2 shows
the end stage that this embryo reached: an essentially normal embryo
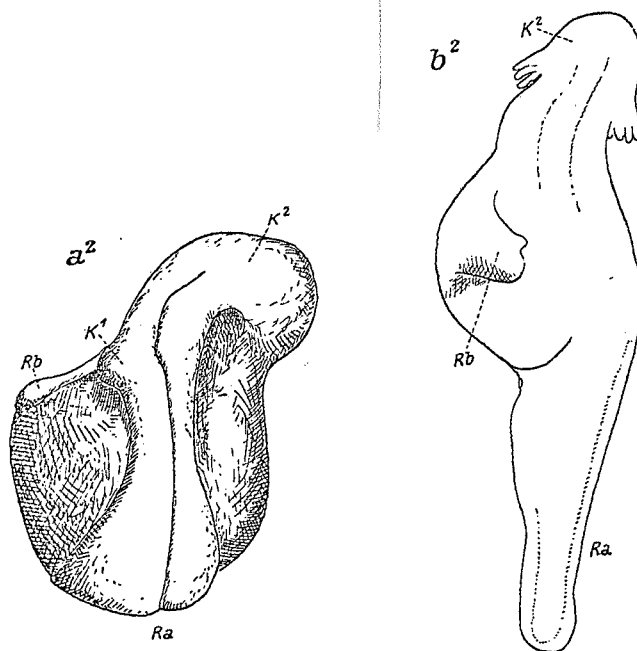
Fig. 99

Embryo IX 7. a^2) 3 April, 10:30 AM,

view from the left. b^2) after fix-
ation on 4 April, 11:00 PM; the same
view.

with already developed gills. A very short little tail which is the
remnant of Rb, visible on the left side, is the only complication.
Nothing more of the rudimentary head from the preceding stages could

be recognized. Nor was any trace of this head rudiment found on serial sectioning. The rudimentary, second dorsum contains no axial organs at all. It consists only of the epiderm and mesoderm (Wittmann). One can conceive of the embryo as a duplicitas posterior since a posterior end which has still not completely retrogressed in the whole form originated from the rudimentary dorsum. There are even transitional forms between this and the single formation. But since the rudimentary posterior end has absolutely no axial organs, we have included this embryo here as a single formation with an appendage.

/85

Eight additional embryos, which became more or less complete single larvae in fully similar manner, will be only mentioned by their control numbers: I 5, V 6-9, IV 18, IX 8 and 9.

SUMMARY

A duplicitas cruciata can subsequently transform into a more or less complete single formation by the progressive reduction, during further development, of the head and dorsum that were initially laid down weakly. An embryo then evolves with a primary head and a secondary dorsum, the median planes of which are exactly or approximately the same, and upon which small appendages representing the residues of the two retrogressed cruciata parts can be located. Fig. 98 presents a schematic picture of an embryo of that sort.

b) Spina bifida, genetically a duplicitas cruciata

In our first report (1925, pp. /132-133) we have already attempted to show that in some final stages of development, cruciata forms can also become more or less single embryos, by the indirect path of a spina-bifida formation. One individual part is then only defectively formed and it is more or less absorbed between the neural folds of the other part, so that an embryo can arise which externally appears to be a primary single formation. In most cases however, it does not become a complete single formation.

/86

aa) Both individual parts differentiate to form an embryo; one is much smaller than the other and forms a kind of yolk plug in the dorsum of the latter.

α) The smaller individual part separates the posterior components of the neural folds of the larger.

Description of embryo IV 12

      A furrow arose dorsally, approximately in the median plane, only a little to the left of it and thus situated paramedially (Fig. 100 a). During subsequent development, this groove became significantly longer and shifted on the whole to the left (Fig. 100 b).
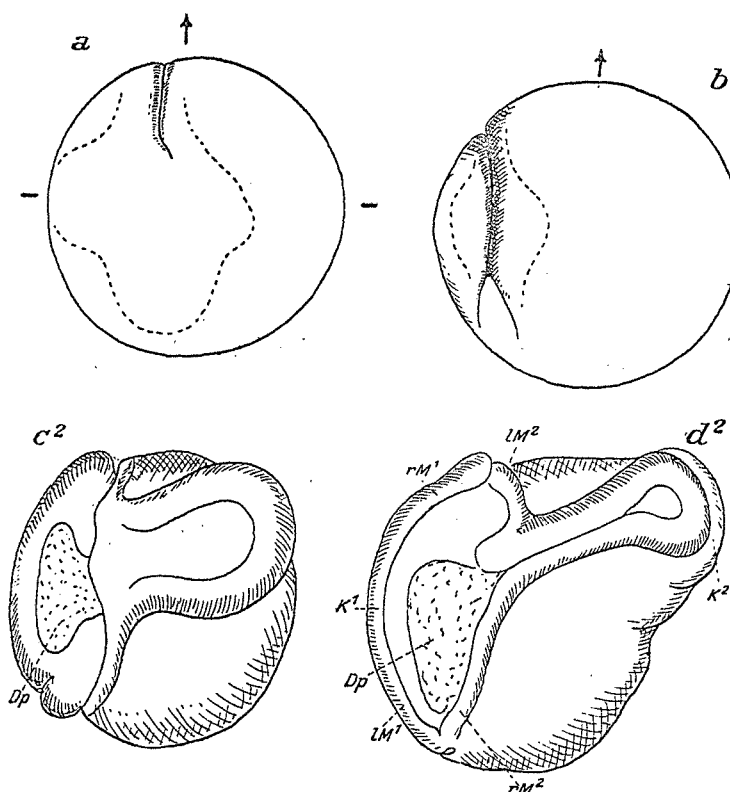
Fig. 100

Embryo IV 12. a) 16 March, 11:30 PM,

b) 17 March, 5:00 AM, c^2) 18 March,

4:00 AM; dorsal view. d^2) 11:00 AM;
the same view. Dp-yolk plug.

Finally, a smaller left half separated from a larger right half.

Fig. 100 c^2 shows the embryo in dorsal view at a very early medullary plate stage. To the right of the original groove on the dorsal edge, a pronounced, long anterior end has been laid down; to the left a complex of white material appears at first which looks like a yolk

plug (Dp). It is surrounded by bulge formations. Fig. 100 d^2 shows the embryo seven hours later from the same view. A long, primarily single anterior end has formed over the large right half of the

embryo that already possesses a clearly defined head (K^2). Along the region of the original groove, to the right of it, the two

neural folds rM^2 and lM^2 grow, forming an angle of almost 180 degrees to each other, as is characteristic of a typical duplicitas cruciata. But this embryo does not represent such a formation. For the smaller individual part on the left displays only a very rudimentary differentiation, specifically, a single neural fold which is stretched in a flat arc between the posterior ends of the neural folds of the individual part on the right. One can, however, consider the whole to some degree as a duplicitas cruciata in the sense

indicated by Fig. 100 d^2. But since the individual part on the right considerably exceeds that on the left in both size and differentiation, one can regard the whole as a single embryo in the form of a spina /87 bifida in which the plug is formed for the most part by a very rudimentary second embryo and consists to only a minor degree of undifferentiated yolk material (Dp).

The final stage of embryo IV 11

Gastrulation was described in detail in Part IV (p. and Fig. 10); it was completed with the formation of a long, almost paramedian groove running somewhat obliquely. Fig. 101 shows the final stage reached by the embryo, in left vegetal view. A distinct head

rudiment (K^2) developed at the dorsal edge, on the vegetal surface, to the right of the original groove. It continues toward the rear

(viewed from the head) in two neural folds rM^2 and lM^2 which have developed to the right of the groove region. To the left of the

original groove, one can see a second head (K^1). It remains externally

visible only to the left (again viewed from the head), where it

terminates in neural fold $1M^1$. The other neural fold cannot be seen. It extends, so to speak, within the interior of the embryo, under

neural fold $1M^2$. The two neural folds which belong to head K^2, extended along the ventral and dorsal edges respectively to the
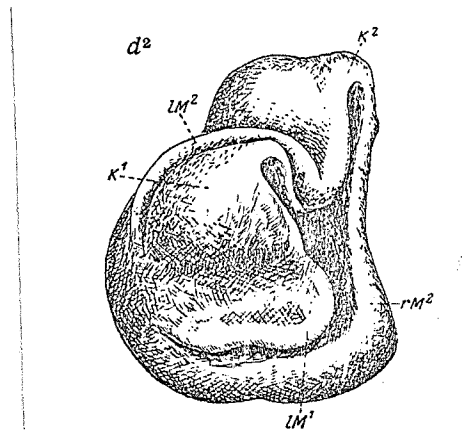
Fig. 101

Final stage of embryo IV 11, the gastrulation of which is illustrated in Fig. 10 (Part IV), after fixation on 17 March, 2:10 PM; left-vegetal view.

animal surface and in quite weakly developed form encircled the embryo which formed left of the region of the groove. Thus, in this specimen the individual part on the right is thus much better developed than that on the left, furthermore the latter sticks as a yolk plug, so to speak, posteriorly, in between the neural folds of the former.

Additional information on other embryos

Two other embryos IV 10 and IV 20 behaved in similar manner as above except that the individual part on the left was better developed than that on the right. In other embryos (IV 15, 18, 19 and
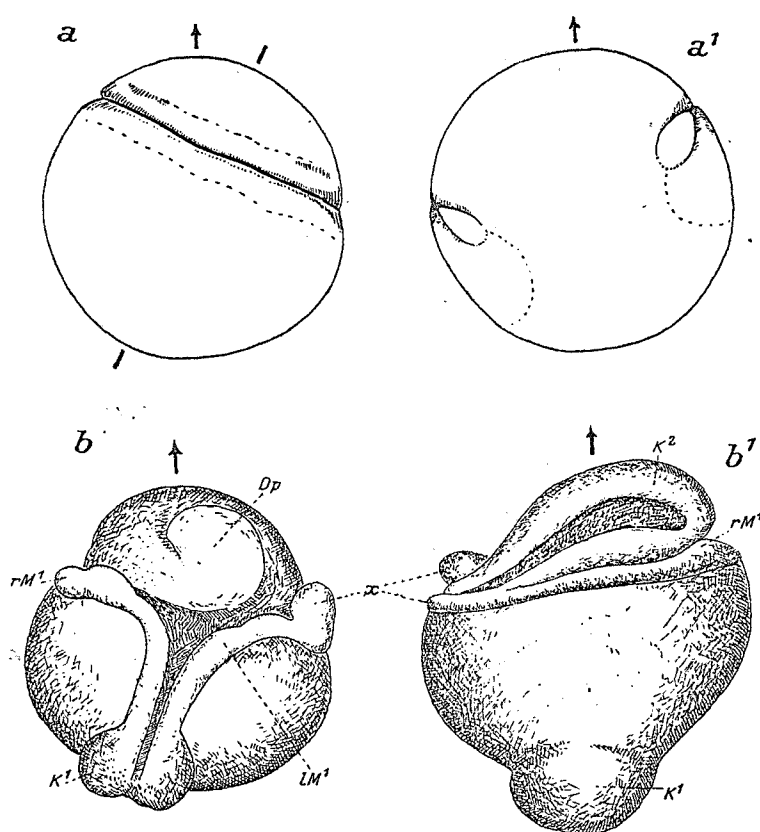
Fig. 102

Embryo IV 18. a) and a^1) 17 March, 1:00 AM,

b) and b^1) 18 March, 6:45 AM. Dp-yolk plug.

II 20) the long groove had a more or less frontal position; in these cases the ventral individual part developed the best.

During such development, very remarkable distortions can occur. Embryo IV 18 may be briefly discussed as an example of this sort.

Figs. 102 a and a^1 show the long groove. It does not lie truly frontally but so that a more dorsally situated half-embryo is separated from a more ventrally located one. The final stage reached by the embryo is shown by Figs. 102 b and b^1 in vegetal and animal views respectively. A long anterior end of primary origin (K^1) has developed over the ventral half perpendicular to the groove and its tip is visible from the animal side: This branches at the rear (viewed from this individual) into the two neural folds rM^1 and lM^1, and these envelop the half-embryo which had developed dorsally to the furrow.

In the animal view (b^1), one sees that the neural fold rM^1 of the ventral embryo extends entirely along the dorsal individual part; it unites with the neural fold lM^1 at the point indicated by x. The dorsal individual part itself is also clearly differentiated. It lies however, completely parallel to the direction in which the groove runs. The entire double formation thus looks very distorted. In addition, another isolated plug of white material (Dp) had upswelled on the vegetal surface in the region of the original groove.

SUMMARY

In all of these formations, a very long groove separated the embryo material into two halves, each of which developed into an embryo. Completely regardless of the position of the furrow in relation to the median plane, one embryo remained a great deal smaller than the other. Either the left-hand one or the right-hand one was the larger or even the ventral one; but the dorsal one never became the larger one, something which we regard as completely accidental. An additional peculiarity of these formations lies in the fact that the smaller individual part always sticks like a yolk plug posteriorly in between the neural folds of the larger one, which thus becomes a spina bifida.
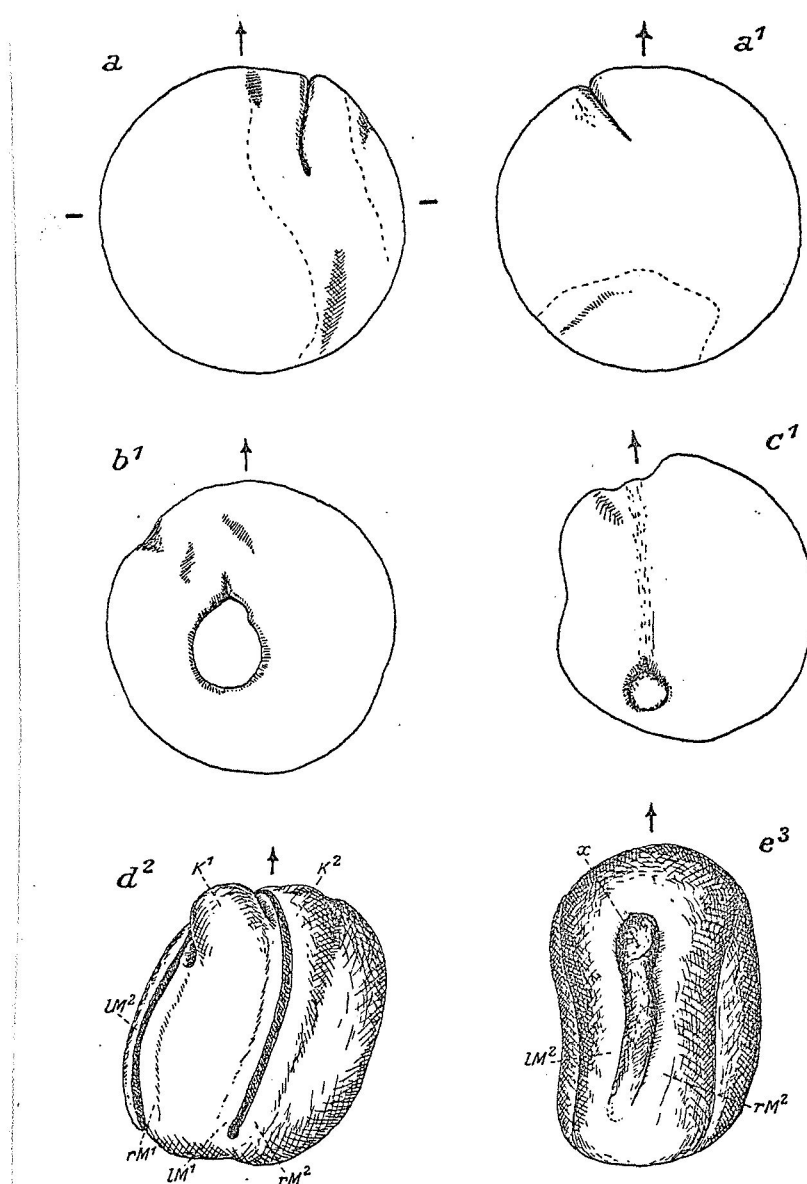
Fig. 103

Embryo IV 21. a) and a^1) 17 March, 12:15 AM, b^1) 10:15 AM, c^1) 3:10 PM, d^2) 18 March, 5:30 AM; right-animal view. e^3) 3:15 PM; right view. x = head k^1.

β) Single formation originating from a typical duplicitas cruciata in the form of a dish-and-lid embryo, through almost complete absorption of the "lid"

Description of embryo IV 21

The embryo gastrulated with the formation of an initially narrow groove. Figs. 103 a and a[1] show it eleven hours after the beginning of gastrulation. A narrow groove is located to the right of the dorsal edge and extends for a considerable distance over both animal and vegetal surfaces. Its closure began vegetally and soon reached the dorsal edge while on the animal end, it lengthened still considerably further and, on the surface, ringed a transparent white

complex like a yolk plug (Fig. 103 b[1]). This complex descended and of the blastopore there then remained on the animal surface at the

ventral edge, a small residue (Fig. 103 c[1]) which persisted until the

embryonic anlagen appeared (Fig. 103 d[2]). The formation represented a cruciata in the form of a dish-and-lid embryo. The margins on both sides of the original groove had bulged into neural folds (rM[2]

and lM[2], and rM[1] and lM[1] respectively); they were somewhat more clearly defined at the dorsal edge than elsewhere and were evidenced

there as faint head rudiments (K[1] and K[2]). Of these, that of the

"lid" was defined the best (K[1]). Development now proceeded so that both neural grooves moved always closer to each other and the "lid embryo" continually decreased in size. The "lid head" shrank to a

tiny button (Fig. 103 e[3] x). The "dish embryo" did not succeed in absorbing the "lid embryo" completely. Prior to this, the latter embryo died. At its final stage, it corresponded to the cases reproduced in Figs. 12 and 13 in our first report (1925, pp. 132-133). It is very possible, that almost completely single formations can arise ultimately from cruciata forms of the dish-and-lid type in this manner.

We still have an entire series of similar embryos, the developmental course of which is not completely clear in the sketches.

SUMMARY

Thus, a cruciata which at first appears typical can subsequently become a spina bifida, and eventually even an outwardly single formation, if the embryo at first appears in the dish-and-lid form and then, during further development, if the "lid" continues to shrink and is submerged into the "dish".

bb) One individual part remains undifferentiated

The forms of spina bifida described up to this point, represented genuine double formations in all cases, in that two individual parts are laid down and differentiated to a greater or lesser degree; namely, these double formations belong in the duplicitas cruciata. In the spina bifida embryos described below, one might at first be in doubt whether it is possible to speak of double formations at all, let alone duplicitas cruciata. For only one individual part is differentiated in these; the other appears at best as an undifferentiated complex of material; but this complex clearly has to some extent a nature of its own in that the differentiated individual part does not utilize it for building itself up. It appears as a separate structure per se and has no real connection organically with the differentiated individual part, or at most only insofar as it imposes the spina bifida character on the latter. Thus, these embryos may also be regarded as double formations. And since the differentiated individual part behaves in all respects exactly like the larger individual part in the spina bifida forms previously described, one might as well regard these double formations also as cruciata forms.

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α) The undifferentiated individual part widely separates the neural folds of the differentiated individual part

Description of embryos IV 17 and IV 16

Gastrulation of embryo IV 17 took place with formation of a long narrow groove. Figs. 104 a and a^1 show the almost circular

groove which ringed the entire vegetal surface and ended on the
animal side, enclosing two yolk plugs. Both yolk plugs had com-
pletely circular form and thus it separated the egg material only
incompletely into two halves of approximately equal size, one dorsal
and the other ventral. Only the ventral half developed an embryo
which is shown in Fig. 104 b in vegetal view. The dorsal half did
not differentiate; it remained an undifferentiated spherical struc-
ture between the two neural folds of the ventral embryo and pre-
vented their fusion into a single neural groove, at least for the
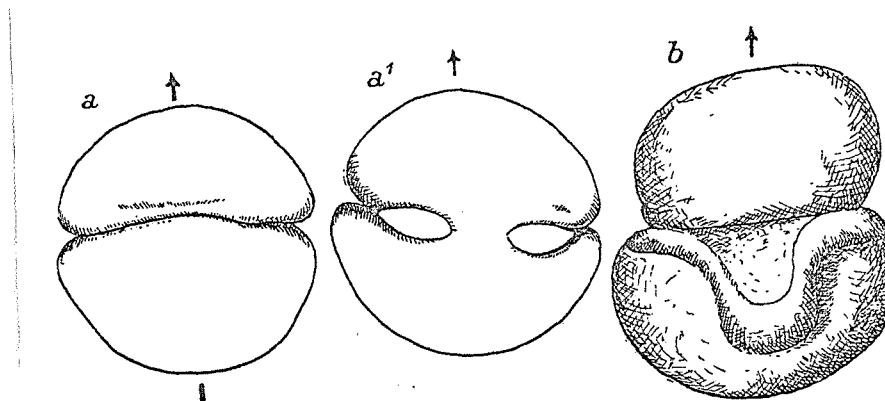
Fig. 104

Embryo IV 17. a) and a^1) 17 March,
5:00 AM, b) 8:00 PM.

most part. Only at the anterior end did fusion take place later.
Afterward the embryo died.

A second embryo IV 16, behaved in principle, in exactly the
same manner. It differed in only two respects from the first.
First, the groove was completely circular and separated the two halves
very deeply from one another and, secondly, it was the dorsal and not
the ventral half that differentiated into an embryo (Fig. 105). This

embryo also was lost soon after the stage represented.

SUMMARY

In these embryos the egg material was divided more or less clearly into two halves by a deep groove (these two halves did not correspond closely to the first two blastomeres however). Of these
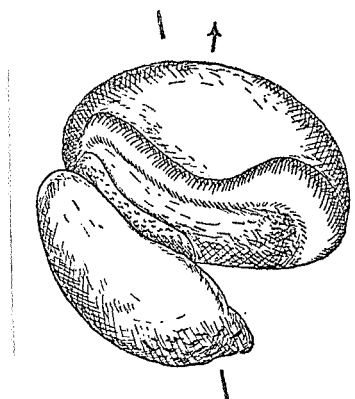
Fig. 105

Embryo IV 16. Final stage shortly before death; vegetal view.

two halves, only one, either the ventral or the dorsal developed an embryonic anlage. The other did not differentiate but separated the neural folds of the differentiated embryo widely so that a typical spina bifida resulted.

In addition, it should be mentioned that the undifferentiated individual part is sometimes significantly smaller than is the case

when both embryos are developed. This was the case, for example, in
embryo I 8. Usually we then are dealing with an embryo that approxi-
mates a single formation in appearance which has a plug of undiffer-
entiated material wedged in between the neural folds near its pos-
terior end.

β) The undifferentiated individual is fully absorbed
by the differentiating part so that outwardly
single formations come into being

Description of the later development of embryo IV 13

Gastrulation was described in Part IV, p. /389 (Fig. 16) in
detail. A long frontally running groove arose dorsally to the
middle of the vegetal surface. In the further course of gastrula-
tion, it closed in the form of a circular groove and migrated as a   /94
whole more and more in the dorsal direction, until it finally sepa-
rated a very small dorsal part from a large ventral part. Since
the circle formed by the groove subsequently narrowed, an apparent-
ly normal, small Ruskon anus developed, in which the originally
dorsal, small part was, however, completely absorbed. Now the
embryo outwardly quite resembled a single embryo (Fig. 106). From
the blastopore residue situated dorsally on the animal surface, a
medullary plate developed which extended over the dorsal edge and
further over the vegetal surface to the ventral edge, closing to
form a neural tube and ending in a head at the ventral edge. The
embryo, which appeared completely normal outwardly, formed gills
after two more days of development and it was then fixed. The ex-
tent to which the absorbed dorsal embryo part is still evident
internally at the final stage attained and the differentiations
formed by it will be discussed by Miss Wittmann.

Additional information on other embryos

Embryo IV 14 exhibited the same developmental course and the
same final stage. In contrast to the embryo described above, in
embryo I 7 a small ventral half-embryo was separated from a large
dorsal half-embryo by the groove. Consequently the ventral half was
also absorbed in this formation while the dorsal one developed into
a single outwardly normal embryo which, in contrast to embryo IV 13,
is oriented normal to the original bilateral line of the egg.
Embryos I 13 and I 8 behaved similarly; in their case, in particular,

the groove simulated a normal blastopore.

SUMMARY

        Thus, in many of these embryos, a long frontal groove ap-
peared. Had this remained in the position where it had arisen, two
embryonic anlagen would really have had to develop, with the head of
the one being oriented from the groove in the ventral direction, the
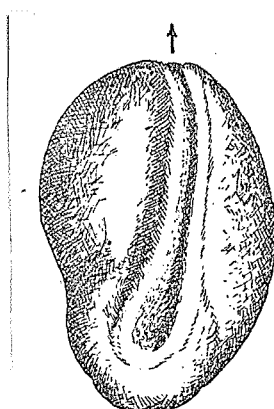
Fig. 106

Final stage of embryo IV 13, the
gastrulation of which is illus-
trated in Fig. 16 (Part IV);
vegetal view; 18 March, 12:30 PM.

head of the other turned from the groove in the dorsal direction,
and both anlagen could have combined to form a duplicitas cruciata.
But in fact, one half-embryo was absorbed by the other already
during gastrulation, that is, it was overgrown as epibolically as if
it had been the vegetal field of a normal embryo and the furrow of a

/95

normal round blastopore lip. On the larger half which ultimately was the only one still visible there developed externally therefore an embryo completely normal in appearance. This embryo is a single embryo of primarily single origin. In both embryos with a seemingly normal blastopore, the blastopore was in reality a groove which had slipped off to one side of the embryo in the manner outlined above.

cc) Summary on the origin of single embryos from spinae bifidae

All of the embryos treated in this Section had the common feature of being spinae bifidae. The degree of this character as well as the nature of the plug, which determines this character, are very different. 1. On the one hand, we see that the plug is formed from a completely undifferentiated embryo material, on the other hand, the plug can also represent a more or less differentiated individual part. 2. The spina bifida character can be manifested to an extremely strong degree in that approximately one-half of the egg material can, in the form of a plug, separate the two neural folds of the developed individual part along almost their entire length. It may also happen, however, that there is nothing more of the spina bifida character to be recognized externally and a seemingly single formation is present, because the "plug" was finally absorbed completely. Between these two extremes, there are various gradations in the size of the plug and this occurs in both series, both in the forms with a differentiated plug and those with an undifferentiated plug. The latter series is represented by embryos IV 17 (Fig. 104 b), IV 16 (Fig. 105), I 8 (no diagram) and IV 13 (Fig. 106), the former series by embryos IV 11 (Fig. 101),

IV 18 (Fig. 102 b and b^1), IV 12 (Fig. 100 d^2) and IV 21 (Fig.

103 e^3). In both series, the plug is the largest in the beginning and the smallest or no longer recognizable at the end. In the series with the differentiated plug, one can recognize the cruciata character of the overall formation, to some degree, with complete clarity at early developmental stages. In the other series, of course, this is not so. Despite this, one must consider these embryos as also belonging to the cruciata forms for they would have belonged to such a category if both the individuals had differentiated. In this latter series, many embryos outwardly are normal single formations. In the other series with the differentiated plug embryo, this is not the case. However, one cannot doubt the possi-

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bility that in this series too, single embryos of outwardly normal appearance can develop. These single embryos are all to be considered as primary single, since they have all been laid down, in their entirety, on one side of the gastrulation groove, hence, from one individual part.
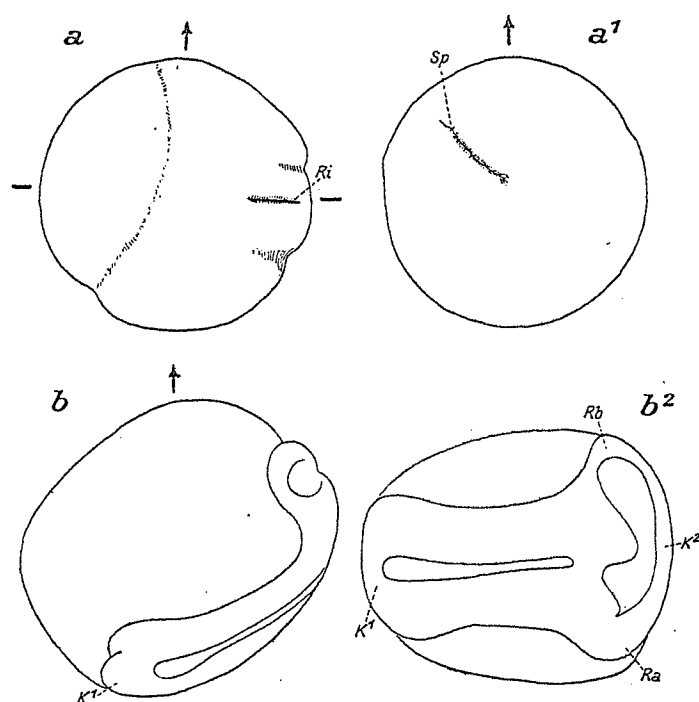
Fig. 107

Embryo VI 21.   a) and a[1]) 24 March, 12:10 PM,

b) and b[2]) 25 March, 7:50 AM; b[2] view from right-ventral.   Ri-groove, Sp-animal blastopore slit.

c) Primary single formation, genetically a duplicitas cruciata, from which only one individual part differentiates and uses completely or almost completely the material of the other for its construction

In the foregoing section b, spinae bifidae have been described which, externally considered, are to be regarded as single embryos. And to be sure, they are primary single formations since in all cases there is only one individual part, which has more or less absorbed the other as a yolk plug. In this Section, primary single formations are also discussed. They originate in a different manner however: for they do not show anything of the spina bifida character at any stage of development. Their dorsum appears quite normal more nearly from the beginning; only their ventral side displays more or less large complications at the posterior end to some degree, since a second individual part can be distinguished there through some differentiations or it may be at least separated to some degree as a complex of undifferentiated material. However, it may also happen that external findings reveal nothing of a second somewhat rudimentary individual part at the stage of earliest embryonic indication. One individual part has then apparently used the entire egg material for its own construction.

α) One individual can still be recognized as a small rudiment

Description of embryo VI 21

After the descent of a small yolk plug on the vegetal surface, a short frontal groove developed there (Fig. 107 a). Similarly, a short oblique blastopore slit developed to the right on the animal surface (Fig. 107 a^1). Probably these two short grooves united in the further course of gastrulation, at the right edge; this cannot be ascertained with certainty from our sketches, however. The embryonic anlage at the open medullary plate stage is shown in Figs. 107 b and b^2 in vegetal and right-ventral views respectively. From the presumed site of junction of these blastopore grooves at the right edge, a medullary plate extends from that edge ventrally, terminating in a head rudiment (K^1) in the region of the median plane. Even the side view of this embryo rudiment (Fig. 107 b) shows that it is not

completely normal at its posterior end, a complication being present

there. Its right-ventral view (Fig. 107 b^2) provides more details
concerning this: A second, somewhat distorted, small embryonic an-
lage, is located approximately transversely to the first, at its
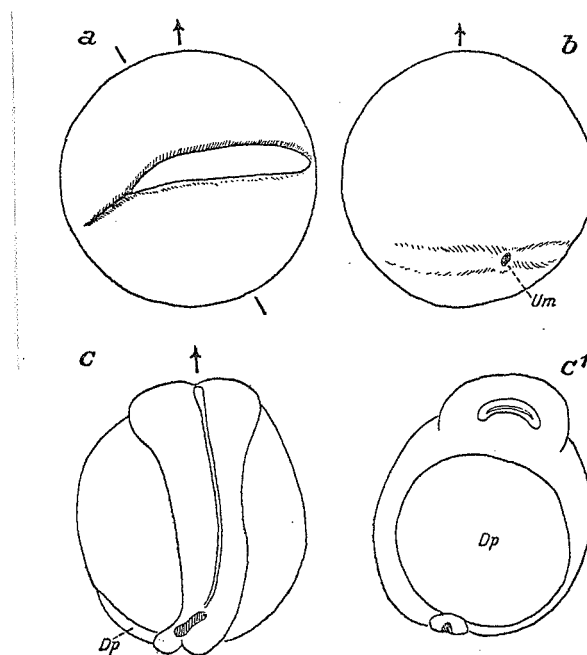
Fig. 108

Embryo I 11. a) 10 March, 4:50 AM,

b) 11 March, 2:40 AM, c) and c^1)
11:30 PM. Dp-yolk plug. Um-blasto-
pore residue.

posterior end. One can even recognize something of the cruciata
character in the entire structure, in approximately the sense in

which we have indicated it by means of the symbols K^1, K^2, Ra and Rb.

Thus, while K^1 and K^2 were on opposite side of the groove, Ra and Rb developed in its original line of orientation. One large embryonic anlage is consequently to be considered with good reason as a primary single formation which only carries at its posterior end, the insignificant remains of the other individual part.

Description of embryo I 11

A broad, approximately frontally located groove appeared here on the vegetal surface (Fig. 108 a). During the descent of the yolk plug, the groove as a whole shifted toward the ventral edge. There it underwent closure until only a small residue was left (Fig. 108 b, Um). In the region where the groove had closed a narrow, faint

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ridge became visible. Figs. 108 c and c^1 show the final stage of this embryo in vegetal and animal views or, relative to the embryo proper, in dorsal and ventral views. For then we are dealing with a single embryo, which extends from the blastopore residue on the ventral lip over the vegetal surface toward the dorsal edge, where the head has formed. One further sees in the vegetal view that the ridge of closure extends to the left as a faint bulge at the posterior end fold, and the animal view shows that on the animal surface this ridge encircles a large plug of material that has not entered into the formation of the ventral side of this single embryo. One must presumably interpret this plug as the undifferentiated second individual part. The other is a complete, primary single formation.

Additional information on other embryos

In both the cases considered up to this point, the single embryo displayed a complication of rather large extent at its posterior end. In a whole series of other embryos, at the stage of the open medullary plate only a tiny button from the second rudimentary embryo was recognizable at the posterior end of the actual single embryo proper. Embryos I 12, III 10, VI 19, IX 21 and X 17 behaved in such a manner, for example. An additional embryo of this kind, IX 13, developed so far that it formed gills. At that stage it was a typical single formation except that to the right of its posterior, it displayed a second, rudimentary tail.

The last embryo, IX 11, in this group, developed to the point of forming a sucker. In this embryo, a broad groove next formed on

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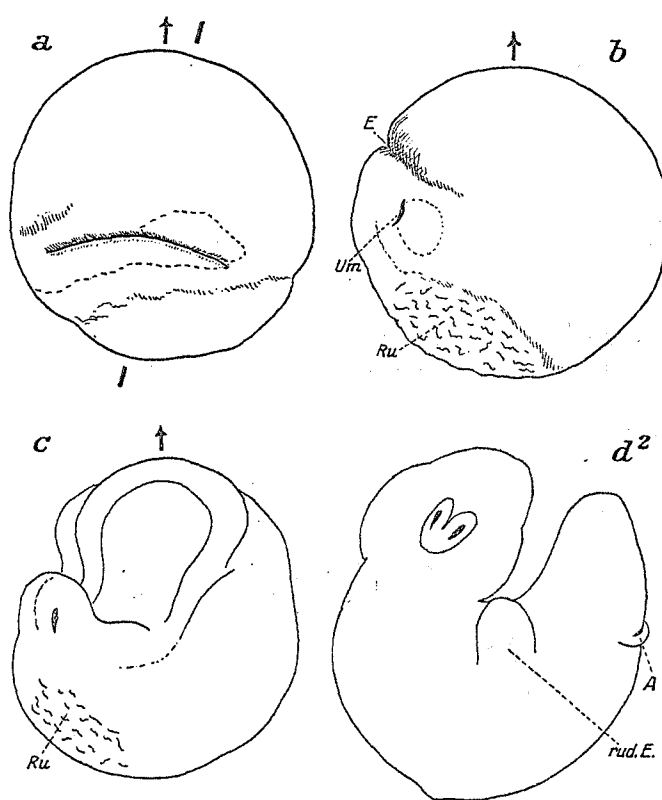
Fig. 109

Embryo IX 11. a) 2 April, 12:30 AM,
b) 3 April, 1:00 AM, c) 10:00 AM,

d^2) after fixation on 4 April; ap-
proximately an animal view; A-anus,
E-depression, Ru-wrinkles, rud. E-
rudimentary embryo, Um-blastopore
residue.

the vegetal surface, and, after the descent of the yolk plug, became
a narrow, frontally situated groove (Fig. 109 a). Ventrally to the
groove, an embryonic region was delimited by a faint furrow forma-
tion; during subsequent development, when the groove closed from
both ends, it became a wrinkle formation (Fig. 109 b Ru). Simul-
taneously, a deep depression (E) arose dorsally to the region of the
original groove. When the embryonic anlagen appeared (Fig. 109 c),
a broad medullary plate extended itself from the dorsally groove
residue over the left half of the vegetal surface. As a consequence
of the previously mentioned depression, this plate was very con-
cavely curved. At the dorsal edge, the medullary anlage terminated
in a distinct head bulge. The entire embryonic anlage had developed
perpendicular to the furrow on its dorsal side. The previously men-
tioned wrinkled complex still remained on its ventral side. This
was obviously the other embryonic anlage, which remained undiffer-

entiated. After the embryo has formed the sucker (Fig. 109 d^2), it
produced a completely normal impression. However, it displayed a
small outgrowth (rud. E) on its left side, somewhat to the front of
the anal region. One must presumably regard this as the second,
rudimentary embryo. In addition, the embryo was naturally curved
inward in extremely concave fashion.

SUMMARY

In all of these cases, therefore, a structure developed which
is in general to be designated as a single embryo displaying at its
posterior end a complication in the sense of a more or less large
appended formation, which is to be interpreted as a second, rudi-
mentary embryo. In a great majority of cases, its size was very
insignificant so that the one developed embryo had, so to speak,
used all of the egg material for its own construction. In addition,
in all cases, these are embryos which gastrulated with groove forma-
tion and in which the one developed individual part was laid down at
right angles to the furrow, on one side of it. It is therefore to
be designated as a primary single formation.
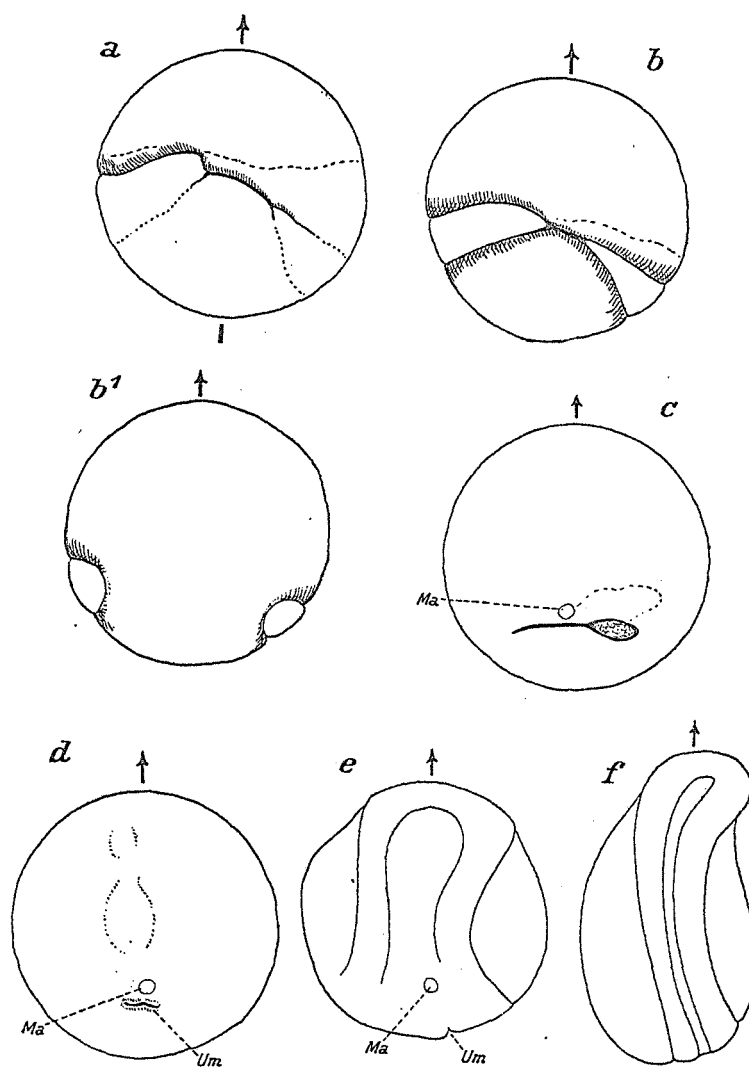
Fig. 110

Embryo I 10. a) 9 March, 1:15 PM,

b) and b^1) 8:45 PM, c) 10 March,
2:20 AM, d) 5:45 AM, e) 3:30 PM,
f) 11 March, 11:30 AM. Ma-random
mark, Um-blastopore residue.

β) No trace of the second individual can any longer be found

Description of embryo I 10

A narrow, frontal groove developed in the middle of the vegetal surface and branched at both ends to enclose two complexes of bright-white material (Fig. 110 a). The groove moved as a whole somewhat further toward the ventral side and the white complexes became smaller at the same time (Figs. 110 b and b[1]). After their descent, the groove began to close from both ends (Fig. 110 c). The groove residue (Fig. 110 d, Üm) shifted toward the ventral edge, where it was still visible at the time of appearance of the embryonic anlagen (Fig. 110 e). A single medullary plate was laid down perpendicular to the groove on its dorsal side, on the vegetal surface. On the other side, there was nothing to be seen of an embryonic anlage. Fig. 110 f shows the embryo approximately 20 hours later. A typical, primary single formation with an almost closed medullary groove is present, since the entire embryo has developed only on one side of the groove, at right angles to it. In this connection, the material which had been situated on the other side of the groove, did not remain as a specifically separate undifferentiated part. Rather, it has completely entered into the formation of the one differentiated individual part. There would appear to be no evidence, from the external investigation alone, for the origin of this single embryo from a cruciata. Sectional examination (Wittmann) shows, however, that it absolutely must be a double formation which belongs to the cruciata.

Additional information on other embryos and summary

Four additional embryos behaved in principle, in exactly the same manner, according to the external findings. All of them gastrulated with groove formation and an embryo was laid down on only one side of the groove, while on the other side, no embryonic anlage developed and the material was used for the construction of the ventral side of the one developed embryo. Two embryos were bent with their dorsal sides strongly concave (III 11 and VI 20). This came about in a manner similar to that which has been previously described for embryo IX 11 (Fig. 109). The other two embryos (I 20 and V 15) were normally formed.

All these single formations are to be designated as of primary origin. Our assumption, that genetically they are cruciatae, is based on the fact, that the embryonic anlage developed on only one
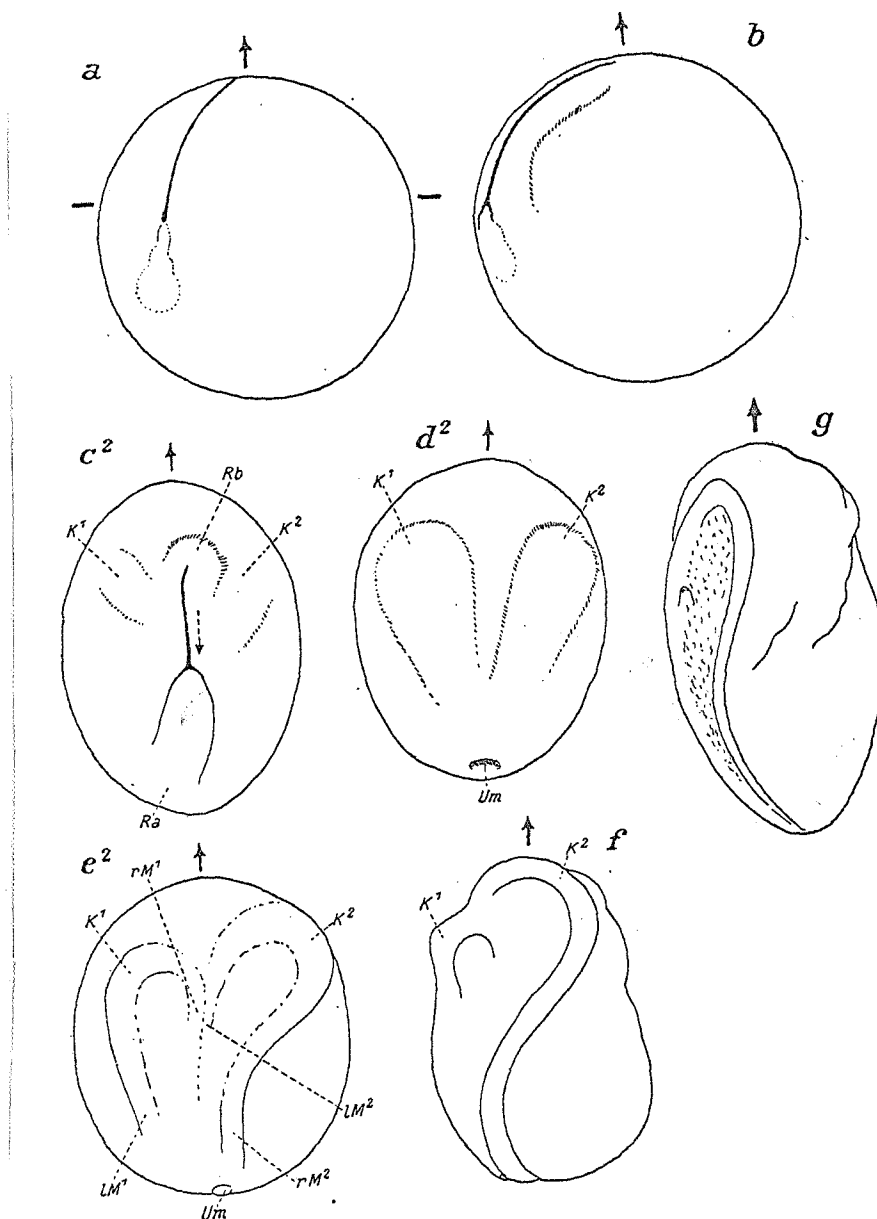
Fig. 111

Embryo I 4. a) 9 March, 4:00 PM, b) 7:25 PM, c^2) 10 March, 12:15 AM;

left-edge view. d^2) 11:00 AM; the same view. e^2) 7:00 PM; the same
view. f) 11 March, 12:15 AM, g) 8:00 AM; Um-blastopore residue.

side of the furrow, perpendicular to it and furthermore, on the evidence provided by embryo I 10, which developed in a similar manner, that genetically it is a double formation (Wittmann).

γ) Summary on these primary single formations

All of the single formations considered in this group arose from embryos, which developed with the formation of a straight gastrulation groove. In such cases, an embryo was laid down only on one side of the furrow, at right angles to it. On the other side, either a very elementary structure appeared or nothing at all that could be designated as a second, rudimentary embryo. All the embryo material on the side of the furrow on which no embryo was formed, was used externally in the construction of the developed embryo so that single formations of completely normal appearance came about, apart from the specifically described complications at the posterior end of the embryos in one sub-group. We can thus with full justification regard these embryos as primary single formations which, however, must be genetically classified as cruciata. This is because, according to the outward course of gastrulation, they would have been able to develop into duplicitae cruciatae, just like all the embryos described in Section V A; and, in fact, one of them can (I 10) demonstrably be traced back to such a form (Wittmann).

d) Secondary unitary formation, genetically a duplicitas cruciata, in which both individual parts develop only one half of the head and one half of the dorsum

α) The cruciata character is still recognizable at the stage of the open medullary anlagen

Description of embryo I 4

Fig. 111 a shows the embryo about six hours after the beginning of gastrulation. A narrow groove had originally developed to the left in the paramedian plane on the vegetal surface and had subsequently lengthened. It shifted as a whole so that it came to lie somewhat obliquely and approached more closely the left edge. Finally, it lay completely at the left edge (Fig. 111 b) and extended from about the

median plane to the middle of the left side. It ended there by en-
circling a bright-white complex like a yolk plug. The groove then

began to close from its dorsal end (Fig. 111 c^2). The plug descended
on the left side; as the embryonic anlagen began to become evident,

a small remnant of it could still be seen (Fig. 111 d^2, Um). One
could already determine at this stage that, on both sides of the
original groove, two medullary plates were developing. Eight hours

later, they were very clearly formed (Fig. 111 e^2). Proceeding from
the blastopore residue to the left edge, two medullary plates ex-
tended dorsally, which abutted against one another in the vicinity

of the furrow with faint inner folds rM^1 and lM^2, while both the

outer folds rM^2 and lM^1 were substantially better developed. They
terminated exactly at the dorsal edge in two head rudiments, which
were also situated beside one another, and of which the inner halves
were significantly less developed than the outer. During further de-
velopment, these two outer head halves combined to form a single
head. The inner halves became increasingly rudimentary, as did the
inner neural folds, and they finally necrotized. As dead material,
they then separated the two outer neural folds from each other (Figs.
111 f and g). These formed, together with the two outer head halves,
a spina bifida, which, if regarded as a single formation, is to be
considered as of completely secondary origin. At the stage shown in

Fig. 111 c^2, something of cruciata character could be recognized, as
indicated by the symbols in the figure.
    One can assume that during the subsequent evolution of the em-
bryonic anlagen, the point at which the two individual parts crossed
moved in the direction indicated by the broken-line arrows (Fig.

111 c^2) and further, that dorsum Rb did not evolve.

Additional information on other embryos

    In other cases, both the outer halves of such cross twins suc-
ceeded in lying down beside one another to form a single embryo
which externally appeared completely or at least approximately
normal, since the inner halves completely retrogressed and their
material apparently was used in the construction of the outer halves.

Fig. 112 a^1 shows embryo II 21 from the animal side with the groove

running in the median plane and enclosing a yolk plug at its end.

At the stage shown in Fig. 112 b^1, one saw a bulge oriented in the direction of the now closed groove, which is to be interpreted as dorsum Rb. Directly to the right of it appeared a medullary plate, clearly the head rudiment K^1. Whether the head anlage K^2 expected
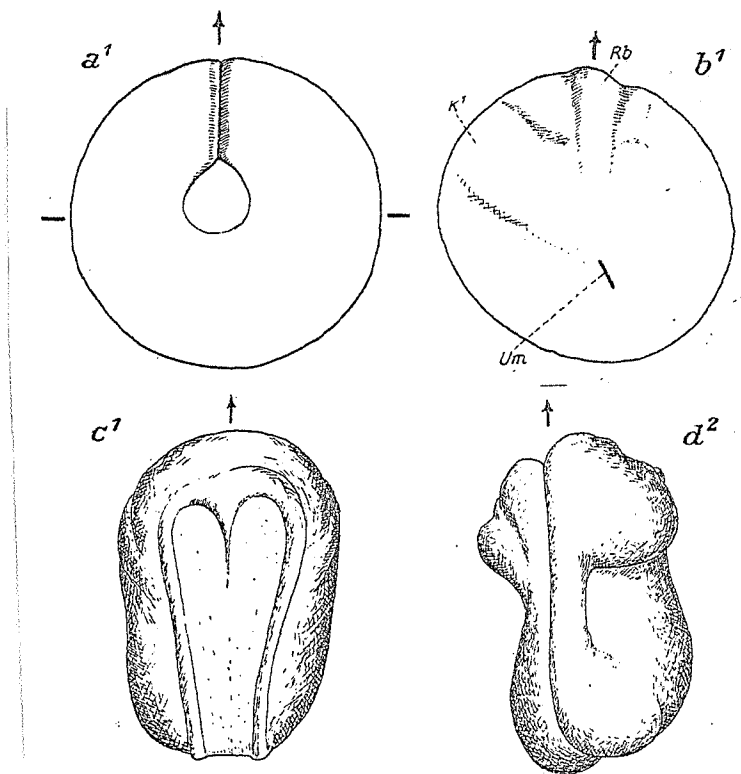
Fig. 112

Embryo II 21. a^1) 13 March, 11:00 AM,

b^1) 14 March, 4:10 AM, c^1) 1:30 PM,

d^2) 14 March, 12:15 PM; dorsal view.

on the left was not present at this time or whether it was overlooked

remains uncertain. In the next stage depicted (Fig. 112 c[1]), apparently a duplicitas anterior in the open medullary plate stage is present. However, a single embryo of completely normal appearance

developed from this (Fig. 112 d[2]). The embryo developed still considerably further; Fräulein Wittmann will report on this.

It can also happen, that such specimens, as freely mobile larvae are not completely normally developed at their anterior end. Thus, in the case of embryo V 11, the eye and mouth regions were obviously not completely normal. A large number of other embryos of similar origin displays the widest variety of tiny protuberances and outgrowths on their anterior end. These will only be mentioned: III 7, I 18 (gastrulation is shown in Fig. 6 of Part IV), IV 23, IX 10 and V 12. They all show, in part even at very advanced stages of development, that they have not passed through a normal development.

SUMMARY

In this group, the embryos, without exception, gastrulated with the formation of a very short groove. The single embryo into which they evolved, came into being in such a manner that on each side of the furrow, a half-anlage formed, one neural fold and a half-head, which combined to form a single formation. The formation of the other components of a typical duplicitas cruciata could be demonstrated from an early developmental stage with a fair degree of certainty. These embryos are therefore to be designated as secondary single formations. One can derive them from a duplicitas anterior-lateralis, as is schematically shown in Fig. 88: First the point of intersection shifts toward one posterior end (Fig. 88 a) and then not only the second dorsum (1, Fig. 88 b) but also the inner half-head (2 and 3, Fig. 88 b) retrogress.

β)   The cruciata character is no longer recognizable
at the medullary plate stage

Description of embryo III 9

A narrow, median groove appeared on the vegetal surface and,
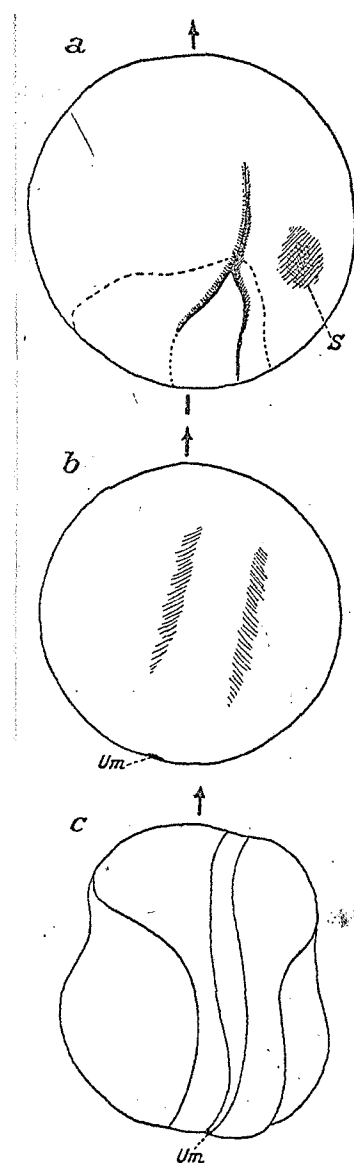
Fig. 113

Embryo III 9. a) 12 March, 10:15 PM,
b) 13 March, 2:15 PM, c) 14 March,
4:15 AM; s-shallow depression. Um-
blastopore residue.

on lengthening in a ventral direction, branched over a bright-white complex and there first began to broaden. It then decreased in length beginning from the dorsal end; the bright-white material descended as a plug on the ventral edge and with that, the groove closed until only a small residue remained (Fig. 113 b, Um); a bulge could be seen above the closed groove. Fig. 113 c shows the formation developed from the embryo at the stage of the open medullary plate. Again, an embryonic half-anlagen formed on both sides of the furrow. The embryo developed still further until the sucker was evolved. This had a form which was not entirely normal. In other respects the embryo is completely normal externally.

Additional information on other embryos and summary

Seven additional embryos developed in the same manner into single embryos and, indeed, some developed so far as to form gills. The orientation of the embryo could be the same as that of embryo III 9, that is to say, normal, thus it was in the case of embryos X 15, 16 and V 14. In the remaining ones, III 4, 5, 6, and 8 the embryo lay on the edge, according to the position which the groove had had formerly, so that the head was clearly located to the right and the posterior end to the left, or the head dorsally and the posterior end ventrally or vice versa.

All the embryos had the same manner of origin: on each side of the groove (this being the form in which the blastopore appeared in all of them) only one-half of an embryo was laid down from the beginning. Nevertheless, we must interpret these embryos as secondary single formations which belong genetically to the cruciata form. For in other cases, the formation of this same groove leads to the development of a typical duplicitas cruciata and in embryos of the above mentioned group α, the undeveloped inner halves of each individual were still recognizable.

γ) Summary on the secondary single embryos

In these embryos, the blastopore appeared in the form of a more or less long groove, which began to close at one end prior to the appearance of the embryonic anlagen and namely, at the point where it had first appeared. It then proceeded to disappear, progressing toward the other end until only a small residue was left. In this case, only one-half of each of the two complete individual parts of a duplicitas cruciata such as should, properly speaking,

have been expected after gastrulation with groove formation, was
formed: On each side of the groove, opposite one another, one neural
fold and one half-head. The parts missing in the cruciata form
either appeared at the beginning of the neurula stage merely as
rudimentary formations which later retrogressed, or else they did
not appear at all. At any rate, single embryos which were complete-
ly or almost completely normal in appearance ultimately developed in
the vast majority of cases. Since they were constructed from two
halves of genetically different individuals, from which they de-
veloped by concrescence along their entire body axes, one must desig-
nate them as secondary single formations. On the other hand, one
must consider them as all belonging to the cruciata, primarily be-
cause of the gastrulation with groove formation but also because,
in some cases, suggestions of a cruciata could be found.

C. POSSIBILITY OF ORIGIN OF THE SPEMANN TYPE OF
DUPLICITAS CRUCIATA IN THE SCHULTZE
INVERSION EXPERIMENT

The embryos described in sections V A and B are all to be re-
garded as belonging to the Schultze type of duplicitas cruciata. It
is characteristic of this type that the heads (anterior ends) are
primarily single and the dorsi (posterior ends), secondarily single
(cf. Fig. 1, Part I, p. /313/). Spemann (1916, 1918, 1919) has pro-
duced a duplicitas cruciata type of an entirely differently consti-
tuted type in Triton, which was later more throughly investigated by
Wessel (1926). Two incipient gastrulae were cut out of the animal
part and then the one vegetal part was united with the other. In
that way, a compound embryo arose which had two dorsal blastopore
lips more or less widely separated from one another, according to
the size of the excised piece, (Fig. 114 a). The material for the
formation of the archenteric roof turned inward about each of these
in the direction marked by the arrows. Both the growing archenteric
roofs may meet and then they must branch (Fig. 114 b). Such an embryo
thus possesses a cross-shaped archenteric roof comprised by two in-   /109/
dividual parts, over which a correspondingly formed region of medul-
lary material differentiates. Since the growing ends of the archen-
teric roofs mark the anterior ends, a duplicitas cruciata with
secondarily single anterior ends and primarily single posterior ends
must evolve. In accordance with Wessel's data (1926), this Spemann
type of duplicitas cruciata is schematically represented in Fig. 115,   /110/
having been adapted to the embryos described below. From a compari-
son with Fig. 1 a (Part I, p. /313/) it is immediately evident that
its constitution is completely different from that of the Schultze
type.
        Naturally, we have not disregarded the question of whether, in
the Schultze inversion experiment, double formations of the Spemann
type could originate. The prerequisites for this might be given:
if, in addition to the vegetal blastopore anlage, there is an addi-
tional, separate animal blastopore slit, as can be observed in a
number of cases. This possibility has already been considered above
(p./49/), but we had come to the conclusion, for the cases described
at that point, that the animal blastopore slit is nothing more than
a continuation of the vegetal blastopore groove, separated from it
by a stretch over which the groove is not recognizable. But now we
have several small embryos which, according to their origin, can be
interpreted with some certainty as Spemann cases; sometimes their
development still shows a complication. We could not achieve com-
pletely certain results for any of these embryos and they should

therefore only be mentioned so that attention would be paid to the appearance of the cruciata forms in question, in further studies of the Schultze double formations.
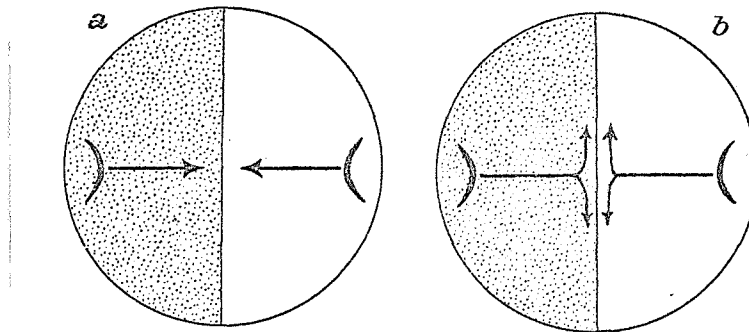
Fig. 114.

Schematic of the Spemann experiment for the production of a duplicitas cruciata in _Triton_. One individual path of the compound embryo is stippled, the other is not; the dorsal blastopore lips are sickle-shaped, the arrows indicate the direction of growth of the archenteric roofs.

1. Formation of a Spemann Cruciata From Primary Ventral Twins.

Description of embryo V 2

A dorsal blastopore lip of approximately normal appearance formed on the vegetal surface dorsal to a very white region (Fig. 116a). This lip developed into a complete circular blastopore (Fig. 116b) which diminished progressively in size, finally closing to become a small slit that moved to the ventral edge (Fig. 116c, Um).
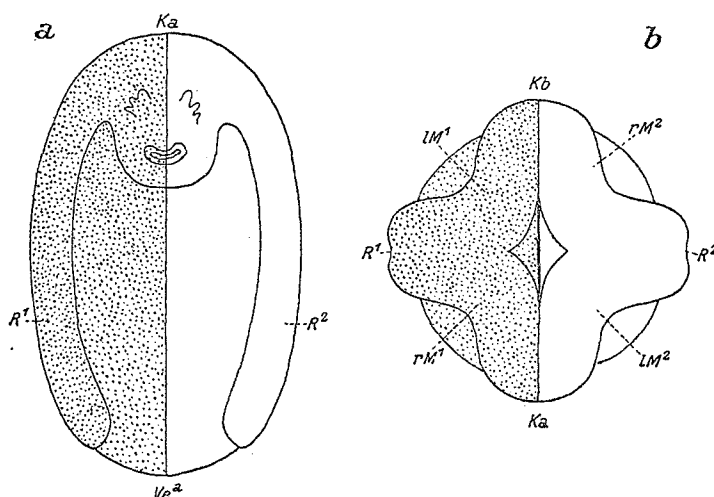
Fig. 115.

Schematic of the Spemann type of duplicitas
cruciata. One individual part is stippled,
the other is not. The two dorsi (posterior

ends) are primary single formations R^1 and

R^2; they divide anteriorly into their neu-

ral folds rM^1 and lM^1, and rM^2 and lM^2 re-
spectively. The two heads (anterior ends),
at which the sucker and gill rudiments are
indicated, are secondary single formations,

Ka comprised by rM^1 and lM^2, Kb comprised by

rM^2 and lM^1. The two ventral sides Ve^a and

Ve^b are also secondary single.

Approximately nine hours later two embryonic anlagen were identifiable. The first was on the vegetal surface and had the already mentioned blastopore residue on its posterior end; its anterior end, formed by the transverse neural fold, extended to the dorsal edge (Fig. 116 d). The second anlage was on the animal side; its posterior end did not reach the ventral edge, but its anterior end did reach

the dorsal edge (Fig. 116 d^1). The transverse neural fold of the vegetal medullary plate constituted at the same time the transverse neural fold of the medullary plate of the animal side. Thus the neural grooves of the two embryonic anlagen were, at this stage,

separated by a wide, transverse bulge (Figs. 116 d and 116 d^1, at x). The vegetal embryonic anlage must therefore be considered as a primary single formation, which originates from the apparently normal blasto- pore of the vegetal surface. We cannot, however, say with certainty how the embryonic anlage of the animal side is to be explained. It cannot have originated from the blastopore of the vegetal surface, since its posterior end did not reach it. Therefore, in the case of this embryo, we assume that an animal blastopore slit was present on the animal surface but was simply overlooked.

From this the animal embryonic anlage may also have arisen as a primary single formation. If this interpretation is correct, then we have here a case of primary ventral twins.

The neural grooves became narrower, the neural folds higher, and the head rudiments, too, became increasingly prominent (Fig.

116 e^2 and Fig. 116 e^3). However, the fold between the two neural grooves, i.e., the medullary material separating them, had become considerably more narrow, as is well indicated by a comparison of

the points marked x in Figs. 116 d^1 and 116 e^3. During the following ten to eleven hours it continued to disappear. In the end the neural groove must have cut across this neural fold from both sides (Fig.

116 f^4, x!), since by this time we had a duplicitas cruciata in the

form of a dish-and-lid embryo (Fig. 116 f^4). One dorsum (R1) occupied the position of the vegetal medullary plate, the other (R2) that of the animal medullary plate. On each side of the median lines of these primary dorsi was a head rudiment (Ka and Kb). These are obviously secondary single formations, corresponding to the

Spemann cruciata type: Ka arose from the anterior ends of lM^2 and

rM^1; Kb from the anterior ends of lM^1 and rM^2.
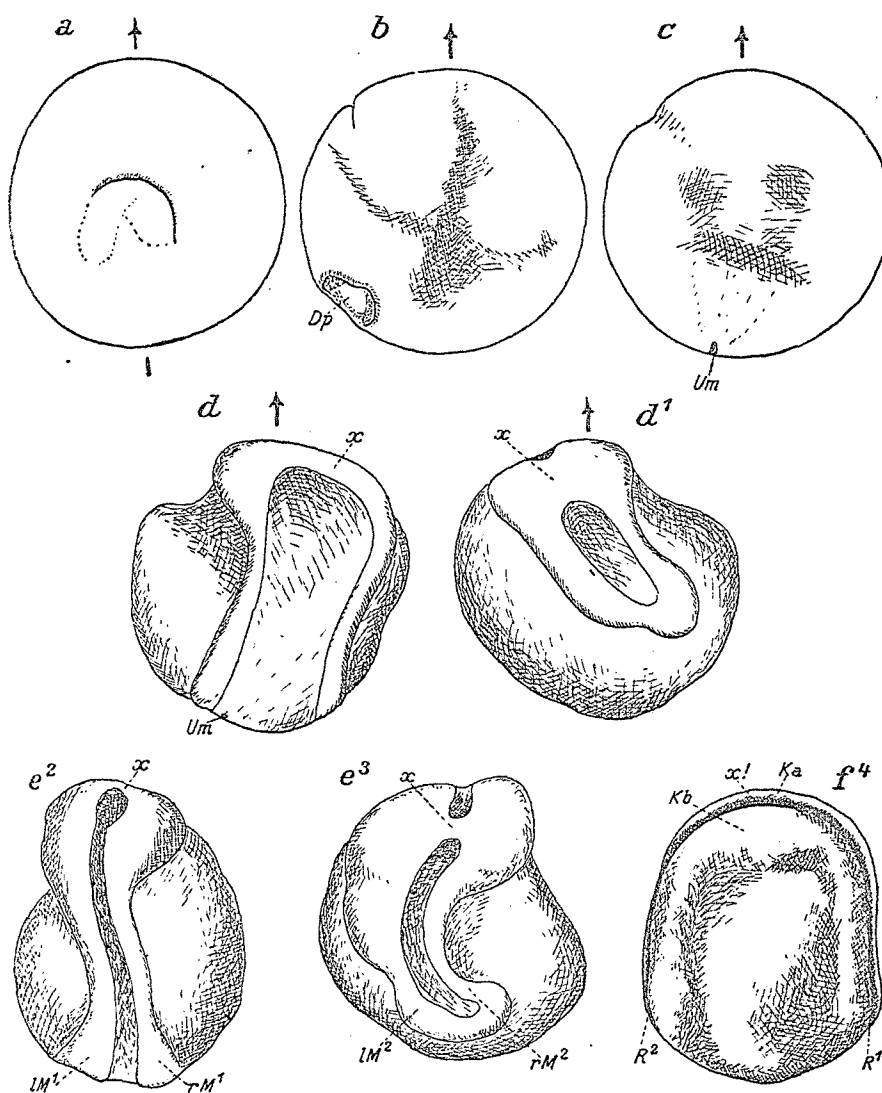
Fig. 116.

Embryo V 2. a) 20 March, 3:45 PM; b) 10:00 PM; c) 21 March, 7:15 AM;
d) and d^1) 4:00 PM; e^2 and e^3) 11:30 PM; e^2 and e^3) 11:30 PM; e^2)
vegetal view slightly from the right; e^3) animal view slightly from
the left. f^4) after fixation on 22 March, 5:00 PM; left view. Dp
yolk plug. Um Blastopore residue, x! point where the two neural
grooves were previously separated by the common neural fold (x).
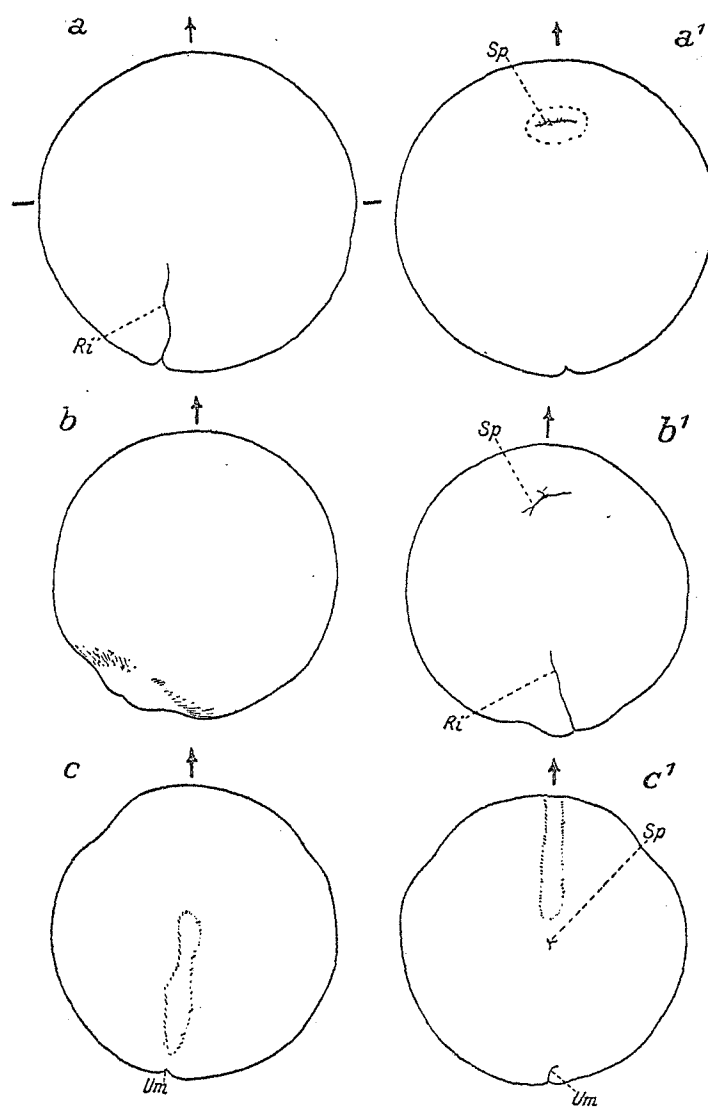
Fig. 117.

Embryo VI 2. a) and a[1]) 24 March, 6:45 AM;

b) and b[1]) 11:15 AM; c) and c[1]) 6:45 PM. Ri
blastopore groove, Sp animal blastopore slit,
Um blastopore residue.

SUMMARY

The above interpretation of the behavior of embryo V 2, is doubtless the most natural, although the lack of proof of the existence of the postulated animal blastopore slit makes it somewhat vulnerable. If it is correct, the development of this embryo corresponds in all essential points to the scheme illustrated in Fig. 114, so that primary ventral twins have turned into a duplicitas cruciata of the Spemann type.

2. More Complicated Mode of Genesis of a Spemann Cruciata
Description of Embryo VI 2

On the vegetal surface, adjacent to the ventral edge, there appeared a narrow groove (Fig. 117 a), which eventually continued around this ventral edge onto the animal surface (Fig. 117 b^1). In addition, a blastopore slit at right angles to the median plane appeared precociously upon the animal surface of this embryo, more precisely, in the middle of slightly whitish material near the dorsal edge (Fig. 117 a^1, Sp). This white material soon disappeared, presumably by involution around the edges of the slit (Fig. 117 b^1). During the further course of development the initial vegetal groove closed until there remained only a small residue at the ventral edge (Fig. 117 c and Fig. 117 c^1, Um). By this time, the animal slit had migrated to the middle of the animal surface (Fig. 117 c^1, Sp). At this stage, two neural grooves were distinguishable: one starting from the groove residue at the vegetal edge and extending to the middle of the vegetal surface; the other on the animal surface, starting from the residue of the animal slit.

If we now assume that the latter continues to the vegetal edge and hence to the residue of the groove, the stage that follows (Fig. 118 d and Fig. 118 d^1) becomes comprehensible: Two embryonic anlagen start out from the two closely adjacent blastopore residues, one on the vegetal and the other on the animal surface, and both extending to the dorsal edge where their anterior ends almost come

into contact. Accordingly, this embryo is to be considered a primary duplicitas ventralis, just like embryo V 2 (Fig. 116).
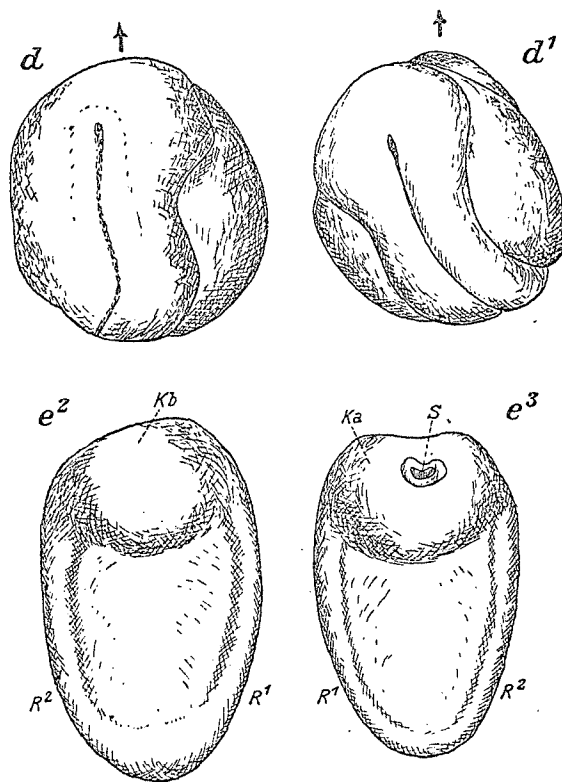
Fig. 118.

Continuation of Fig. 117. d) and d[1])

25 March, 5:00 AM, e[2]) and e[3]) 26 March, 12:00 noon; left and right views, respectively.

In the course of further development, the neural grooves became completely closed and we then got a typical duplicitas cruciata, more precisely one of the Spemann type (Fig. 118 e^2 and Fig. 118 e^3):

Both dorsi $\underline{R}^1$ and $\underline{R}^2$ are primary and correspond respectively to the posterior parts of the vegetal and the animal medullary plates of Fig. 118 d and Fig. 118 d^1. The heads $\underline{Ka}$ and $\underline{Kb}$, the former with a sucker, lie crosswise with respect to the dorsi. Exactly as in the case of embryo V 2, the heads have each arisen from two halves of the two original anterior ends of the medullary plates.

In this interpretation, however, we have left one important point unconsidered. To this we shall now turn. The animal blastopore slit ($\underline{Sp}$), situated at right angles to the median plane, might well have been the cause of the formation in a dorsal direction of an archenteric roof, and hence of a single medullary plate. However, whether enough archenteric roof could possibly turn inward about the dorsal edge of so small a slit as to render the large animal embryonic anlage plausible must be regarded as highly questionable. Much narrower is the groove which wraps around the ventral edge (Fig. 117 a and Fig. 117 b). In this case, as in the case of all such grooves, involution doubtless occurred perpendicular to the edges of the groove, so that an archenteric roof must have been formed to the left and to the right. If this is so, the vegetal embryonic anlage must be considered a secondary single formation of the kind dealt with in Section V B 4d.

The sectional examination of this embryo undertaken by Fräulein Wittmann has not resulted in much further clarification. Where the heads assumed to be secondary occur in the surface configuration (Fig. 118 e^2 and Fig. 118 e^3, $\underline{Ka}$ and $\underline{Kb}$), in the interior we find undifferentiated tissue that may possibly be cerebral matter.

SUMMARY

The interpretation of the development of embryo VI 2 as set forth above is doubtless highly hypothetical. Any other interpretation would, however, be even more difficult to substantiate. Should our interpretation be correct, then the diagram of Fig. 119 a would illustrate the gastrulation process: The vegetal half of the embryo (on the left in the diagram) is divided into two individual parts

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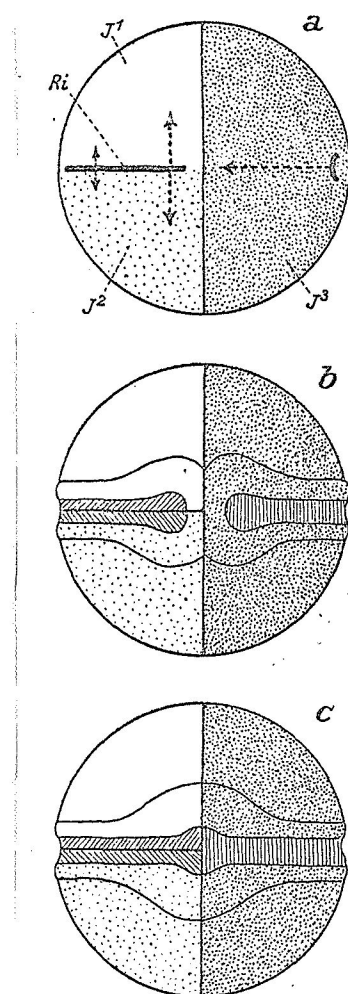
Fig. 119.

Schematic interpretation of embryo
VI 2. Ri Blastopore groove. The

three individual parts J^1, J^2, J^3,
are densely dotted, sparsely dotted,
and not at all dotted, respectively.
The neural grooves are cross-hatched;
further explanation in text.

J^1 and J^2 by the groove (Ri). The archenteric roof is involuted on both sides of the groove at right angles to its edges. It is broad at the dorsal end but becomes narrower over the rest of the groove (as shown by the broken-line arrows of different lengths). The animal half of the embryo (on the right in the diagram) represents a

third individual part, J^3. In this part the involution is around the dorsal edge of the animal slit, in a dorsal direction (see arrow). Fig. 119 b shows the resulting embryonic anlagen: the animal anlage (in the diagram the one to the right) is a primary single formation; and the vegetal anlage (in the diagram the one to the left) is a secondary single formation. The two are in contact at the anterior ends. Fig. 119 c indicates how the neural grooves of the two embryonic anlagen fuse by cutting through the transverse neural fold. In this manner a Spemann cruciata type is formed. This embryo, however, is characterized by the fact that only the animal dorsum (on the right in the diagram) is a primary single formation, as in the Spemann and Wessel cases; the other dorsum and both heads are secondary in nature.

If this interpretation is correct, embryo VI 2 is composed of three individual parts, and thus genetically a triple formation. In the course of our entire study only once was a triple formation detected; it was mentioned briefly in our first communication (1925), but neither its genesis nor its structure was more closely ascertainable.

We found five additional embryos (VI 1, 3, 22 and 24, and XI 4) which also with some justification, but with less certainty, can be considered Spemann cruciatae (in accordance with the diagram in Fig. 114). We wish once again to emphasize explicitly that we consider the interpretation of the embryos set forth in this Section to be strictly hypothetical. We must await the outcome of further investigations of Schultze double formations, before we can decide whether cruciatae of the Spemann type are indisputably present. It is certain, however, that compared with the Schultze type, they are very rare.

D. PRIMARY SINGLE FORMATIONS AFTER APPARENTLY
NORMAL GASTRULATION

Description of embryo I 15

    This embryo, a blastula, was very white in the ventral part
of its vegetal surface, gray-white in the middle region, and dorsally
quite dark (Fig. 120 a). The gastrulation commenced in completely
normal fashion at the boundary between the very white and the gray-
white material. Furthermore, the blastopore groove also behaved
essentially completely normally. It first bent into a U shape (Fig.
120 b), and then closed to form an oval, which embraced the complex
of very white material (Fig. 120 c; the ventral edge of the blasto-
pore lies on the animal side). Normally the yolk plug, surrounded by
the now circular blastopore, lies at the vegetal pole. Here, how-
ever, during its development the blastopore shifted to the ventral
edge, where it became progressively smaller (Fig. 120 d) and finally
shrank to a small slit completely equivalent to the residue of a
normal blastopore (Fig. 120 e, Um). At this stage we observed the
first indication of the medullary plate, which developed in a com-
pletely normal manner in the form of a simple, single embryonic
anlage and subsequently never visibly deviated from the normal
pattern. A normal single embryo resulted without any complications.
(Incidentally, note the gray-white area in Fig. 120 c (outlined with
a broken line) which did not participate in the involution; its
changes in form up to the stage depicted in Fig. 120 f very beauti-
fully illustrate epiboly.)

Comparative

    The development of four additional embryos (I 14, II 23, V 16
and X 18), which in all essential features resembled that of the
embryos described above, has also been experimentally recorded. For
all five embryos (and for many others which, however, we excluded
from consideration even while selecting the experimental specimens),
the inversion experiment did not lead to any important displacement
of the very white yolk. The latter continued to occupy a large area
of the vegetal surface, although in most cases it was no longer
centrally located. One may, therefore, say that in the case of
these embryos the inversion experiments was a failure, so that normal
development to a single embryo had to set in. There is also no doubt
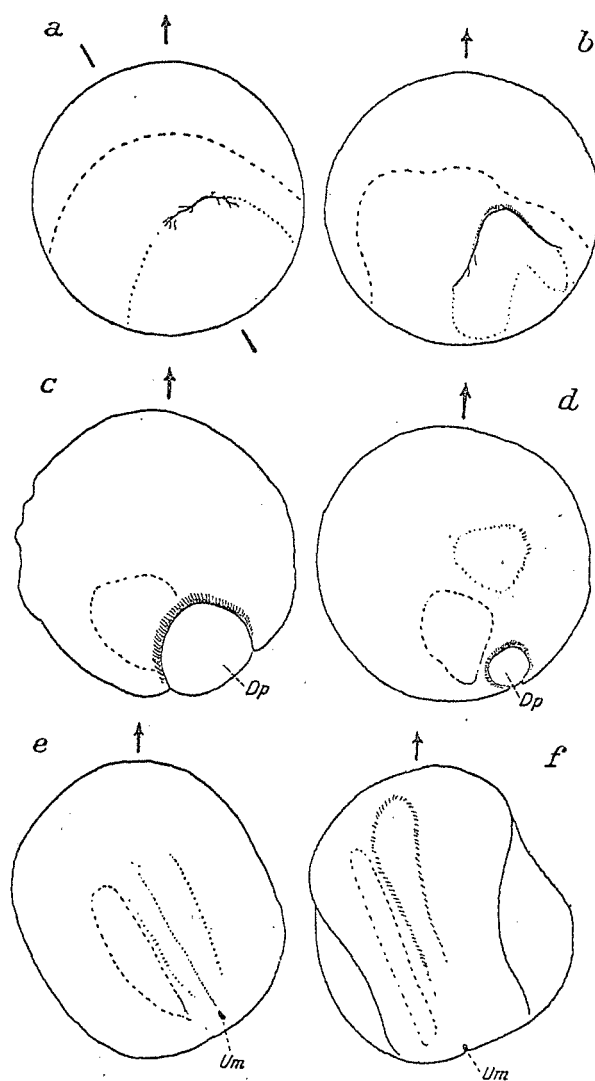
Fig. 120.

Embryo I 15. a) 9 March, 10:30 AM,
c) 10 March, 4:45 AM, b) 9 March,
3:20 PM, d) 10 March, 10:00 AM, e)
10 March, 7:25 PM, f) 11 March,
2:00 AM. Dp Yolk plug, Um Blasto-
pore residue.

that the result of the development of these embryos is in fact a primary single formation, at least insofar as we disregard the possibility that sectional examination might reveal something different.

We have, however, also discussed embryos whose gastrulation likewise seemingly proceeded normally, but which nevertheless finally evolved into a duplicitas cruciata (e.g., X7, Fig. 74). Hence it follows that an identical and outwardly apparently quite normal gastrulation may be associated with either a normal primary single formation or a double formation.

In the case of gastrulation with groove formation we mostly get a cruciata, although in some cases a primary single formation may evolve, with the traces of a second embryonic anlage occasionally found on its posterior ends. This indicates, in our opinion, that genetically a cruciata form was present, but that only one individual part developed, because, in spite of a groove which was to all appearances typical, archenteric roof development was confined to one side only. Thus, outwardly identical gastrulation (in the form of groove formation) does not prove that internally gastrulation is the same. Embryos with outwardly apparently normal gastrulation should behave likewise, either forming only one archenteric roof, as normal, or forming two.

It would, therefore, not be illogical to consider the present embryos with an apparently normal gastrulation, which have developed into primary single formations, as, so to speak, the final stage of retrogression of the cruciata development. In the last analysis, this rests on the internal conditions created by the inversion experiment, namely, on the displacement of yolk. If this displacement is considerable, we get the gastrulation typical of inverted embryos in the form of groove formation. If the displacement of the white yolk was less successful, the latter largely remained hanging to the vegetal surface, and an apparently normal gastrulation follows (as explained in Part IV A). However, the white yolk may nevertheless have been so much disturbed that involution and archenteric roof formation occur in two different directions; at its edge then a cruciata will still evolve. In the case of a still smaller displacement of the white yolk, involution remains restricted to the side which it would normally appear, so that only one archenteric roof, and consequently a primary normal single formation, results.*

---

*We were mistaken in reporting earlier (1925, p. 137) that single formations which genetically are not cruciatae could also evolve from blastulae with a white band.

<u>E. SUMMARY AND CONCLUSIONS</u>

<u>1. Types of Double Formation</u>

Derivation of the Various Forms of Double Formations
From the Schultze Cruciata

Detailed investigation of many inverted eggs from the beginning
of gastrulation until the point where the embryonic anlagen are clear-
ly expressed has shown that actually only the duplicitas cruciata ap-
pears in the Schultze experiment. Although,in full concurrence with
all previous investigators, we too found that the development of the
inverted eggs presents an almost endless variety in the forms assumed.
Out of the 129 embryos whose development we were able to analyze in
the Spring of 1926 (see Table I, Part I), 124 had with absolute cer-
tainty either achieved the final stage, or were at least genetically
to be considered duplicitas cruciatae. Only five embryos proved
exceptions to the general finding: They developed into single for-
mations after what appeared to be largely normal gastrulation, with-
out having displayed at any stage in development definite character-
istics of a genetic cruciata (Figure 120). However, as explained
above, these embryos can be regarded as the last link in a retro-
gression series from genetic cruciatae to single formations. The
cruciata character which in the case of the overwhelming majority of
inverted embryos (one might say in every inverted embryo insofar as
a sufficiently complete displacement of the yolk has been achieved)
is genetically recognizable in the stages of gastrulation or at the
beginning of the definitive formation of embryonic anlagen, is
frequently more or less lost in the course of further development.
The reason for this is most probably that the four constituent parts
of the duplicitas cruciata, two heads lying in one plane and two
posterior ends forming a cross only in rare instances are laid down
with approximately the same vigor and proceed to develop at a similar
rate. Most often one or two constituents of a cruciata formation
are only feebly manifested from the start or else soon fall behind
the others in their development. Fusion of parts and retrogressions
may also occur. Thus it is that we come to observe a huge variety of
transitional forms between typical cruciatae, on the one hand, and
Duplicitates posteriores, D. anteriores-laterales, D. ventrales, and
single formations, on the other. In this connection it is almost
always a question of the duplicitas cruciata of the Schultze type,

which is characterized by two primary single anterior ends and two
secondary single posterior ends (Fig. 1, Part I). Only in very few
instances (Figs. 116-119) did we come, with certain reservations, to
the conclusion that we might be dealing with a cruciata formation
of the Spemann type, that is, with double formations with secondary
single anterior ends and primary single posterior ends (Fig. 114 and
Fig. 115); at least this seemed to us the simplest and most plausible
explanation of their mode of formation.

 We have designated modified cruciata embryos those formed
ontogenetically as Schultze cruciatae but in the course of further
development have turned into another double formation or into a
more or less single embryo. Such modified forms can arise from
typical cruciatae, if the cruciata components wholly or almost uni-
formly laid down during the medullary plate stage partially retro-
gress or if fusion of parts occurs. Such Schultze cruciatae, in
which even at their first appearance individual parts of the Janus
formation are laid down only incomplete or relatively small almost
invariably develop into modified forms. Thus, we obtain:

 1. Posterior duplications, in which one of the two cru-
ciata heads becomes rudimentary (see page /50). This can happen in
two ways. Thus, in the case of a cruciata of the "dish-and-lid"
embryo form, where the two neural grooves of the posterior ends form
not a straight angle but one considerably smaller, the "lid head"
may remain rudimentary (Figs. 76 to 78). However, it can happen
that in a cruciata in which both the neural grooves of the posterior
ends are located in a meridional plane, i.e., form a straight angle
with one another, one head may be weakly laid down from the very be-
ginning, retrogress increasingly in the course of further development,
and finally to all outward appearances disappear completely (Figs.
79 to 81).

 2. Anterior duplications which might also be lateral twins
(see page /60). These occur when one of the two dorsi of a genetic
cruciata is not properly developed (Figs. 82 to 85), or when one
dorsum is to a certain extent folded back on the other and remains
rudimentary. In the latter case the point of intersection, geneti-
cally "normally" located, is displaced more and more toward the end
of the dorsum that still continues to develop. In this way it is
possible to get double formations whose axial organs are almost
totally separated from one another, so that they must be classed as
lateral twins (Figs. 86 to 88).

 3. Ventral twins (see page /70), and, first of all, those
which must be designated secondary because their dorsi and tails

represent secondary single formations, the halves of which belong
genetically to two different individual parts (see page /70, also
Figs. 68, 69 and 95I). These twins originate in genetically typical
cruciatae in which the point of intersection lies very far anterior-
ly and the two primary head rudiments subsequently fuse to form an out-
wardly apparently single head. Secondly, however, primary ventral
twins may also occur (see page /71) if the short secondary posterior
ends of a genetic cruciata retrogress. There then remain only the
long primary anterior ends and it is a question of double forma-
tions whose two individual parts meet at the posterior ends (Figs.
89-94, 95II and 95III).

4. Single formations, which may arise from cruciatae in
various ways.

a. A head and a posterior end of a cruciata are laid
down only very feebly from the start, and in the course of further
development undergo more or less severe retrogression (see page /80).
Embryos with small appendages but otherwise very much the same as the
single embryo may thus be formed. The anterior end of a formation
of this kind is genetically primary single, the posterior end
secondary single (Figs. 96-99).

b. The Wetzel form of the duplicitas cruciata can be re-
garded as two spinae bifidae linked together in such a way that one
individual part is inserted in the dorsal slit of the other, and vice
versa (Fig. 1b). Then each individual part develops a head and the
axial organ halves of a dorsum, which, because of the spina bifida
character, can not grow together. Such a formation can now give rise
to a single embryo with a spina bifida character if one individual
part is differentiated and the other one is not (Figs. 104-105); or
if the second part undergoes a certain, but very small differenti-
ation (Figs. 100-103). In this case the spina bifida character may
be very strongly developed if the two neural folds of the individual
part that either was the only one to develop or at least became very
much the larger are separated along almost their entire length by
the second individual part acting as a "plug." It does happen,
however, that if the plug embryo is absorbed between the neural folds
of the other then outwardly there will be nothing to indicate a
spina bifida character. Then, to judge by the external findings,
the embryo is a single one, and, in fact, a primary single embryo,
since its axial organs have arisen from only one individual part
(Fig. 106).

c. Such primary single embryos can also arise in other

ways (see page /96): For example, embryos often develop a straight 180° groove during gastrulation; an embryo, however, becomes laid down only on one side of this groove, roughly perpendicular to its middle region; on the other side there is no embryo, or only a rudiment. The axial organs of such a single formation are primary single, i.e., originate in a single individual part (Figs. 107-110).

d. There are also other instances of gastrulating embryos forming a straight groove (see page /103). The one embryo finally differentiated, however, lies in the direction of the groove and in its original area, half on one side of the groove and half on the other (Figs. 111-113). Thus its axial organs are secondary single and derived from two different individual parts. The fact that genetically the egg material is actually divided into two individual parts is not only indicated by the groove formation but to some extent is also clearly recognizable in that in some cases a duplicitas cruciata first becomes laid down only after the groove, starting from one end and progressing to the other, had closed. The duplicitas cruciata had only subsequently evolved into a single formation, when the point of intersection shifted to one posterior end and the other posterior end and half the head of each individual part became atrophied.

O. Schultze (1894) has described a double formation of a special kind. He reports that he once obtained a double formation in which the axial organs of the two embryos were totally separated from each other and lay upon the common yolk mass with the head of the one adjacent to the posterior end of the other, and vice versa. We have still to observe formations which correspond fully with this description, but we have several times had the opportunity to observe embryos, undoubtedly of cruciata form, the axial organs of which were so much distorted that in view of our insufficient knowledge of their mode of origin we might have arrived at an interpretation similar to that of Schultze but certainly incorrect.

Reindividualization of Double Formations

A general survey of the various cases of modified forms reveals that there are many transition forms between a typical duplicitas cruciata, on the one hand, and other double formations and single embryos, on the other. In the individual groups we chose to start from forms which at first glance belong among the

typical cruciatae. Then we dealt with forms which, in their end
stages, deviate increasingly from the latter, until finally, at least
to judge from outward appearances, the form just considered (D. pos-
terior, D. anterior-lateralis, D. ventralis, single formation) was
more or less purely expressed in the end stage. From these transi-
tion series, especially those that relate to single formations, it is
possible to conclude that in the development of the egg there is an
innate tendency to form a single whole, even if by artificial in-
tervention a development is imposed which actually must lead to a
double formation. If the development of the individual embryos
described above is examined more closely, this impression is re-
ceived particularly forcefully. In particular, with a series of
embryos, which outwardly considered finally produced a single
formation, one can demonstrate very beautifully how they arose from
an initially plainly established cruciata by processes of retro-
gression and transformation affecting individual parts. Embryo
II 19 (Fig. 98), for example, at first displayed the four components
of a cruciata in the typical cross arrangement, except that one
posterior end and one anterior end were considerably smaller than

the other parts. A primary anterior (K^1) and a secondary posterior
end (Ra) were laid down from the start as a main head and a main
dorsum, respectively, but -- and this is of special interest to us --
these were oriented at right angles to one another, in accordance with
the cruciata character of the formation as a whole. In the course of
further development two things could be established quite clearly:
The main head and the main dorsum extended in one direction, while
the two rudimentary parts became increasingly stunted, until they
finally formed only a small lateral appendage on the "single embryo."
What is in principle the same thing was achieved by other means in
connection with, for example, embryo II 21 (Fig. 112), genetically
a cruciata which, however, finally gave a highly perfect secondary
single formation. In this case of the two individual parts of the
established cruciata only one-half became differentiated, the other
retrogressing.

Thus, in our inversion experiments we often found that an
unmistakable genetic double formation evolved into a single embryo,
a phenomenon recently discussed by Steinmann (1917) under the heading
"Reindividualization." Steinmann has demonstrated this particularly
with planarians, artificially transformed into double formations by
transsection. On this occasion, however, he also reported quite
briefly a case of reindividualization connected with a genetic double
formation, namely in a trout. This concerned an embryo with two
heads, one of which, characteristically, as Steinmann emphasizes,
was of natural size, while the other was very small and rudimentary,

and attached laterally to the first like a growth. As in our case II 19 (Fig. 28), in this trout embryo the main head eventually oriented itself in the direction of the common body. This head grew in size, became more and more differentiated, while the small secondary head became ever more minute. However, before the latter became completely reduced, the embryo died, which, according to Steinmann, generally happens at a relatively early stage in such cases of malformation in the trout. In this case, therefore, re-individualization did not completely succeed. The same is true of the double formations obtained in the inversion experiment. Mangold and Seidel (1927) describe similar processes, which they observed on their fusion embryos of urodeles; and Goldfarb (1915) made thorough examinations of the reindividualization of double formations which he obtained from fusion embryos of sea-urchins.

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Triple Formations

Wetzel (1896, pages 21 ff. and Figs. 9-14 of Plate II) has described a triple formation, but only the final stage it reached. According to this description, it was a duplicitas lateralis, that is, two embryos lying side by side, with a third, very small embryo on the common ventral surface. We too once obtained an unquestionable triple formation (see Schleip and Penners 1925, page /135). For Wetzel, the genesis of such formations necessarily remained an enigma, because he thought that the more or less isolated development of the first two blastomeres was responsible for the double formation. In the light of our theory of the causality of the process of gastrulation, however, the origin of triple formation presents no difficulties. Since any part of the surface of a blastula, originating in an inverted egg, can become an upper blastopore lip, if it comes in contact with very light white material, it is even very probable that in addition to the anlage of the duplicitas cruciata, one or several other anlagen should begin to form. In fact, it is difficult to explain why such multiple formations do not occur more frequently. However, we may assume that the two involution lips, which develop more strongly merely by accident and lead to the formation of the cruciata, can exert a stronger, more energetic influence, in Goerttler's (1927) sense, causing any other weaker anlagen that may be present to disappear. It is possible that even in the course of our experiments such supernumerary anlagen often temporarily appeared and were simply overlooked. We might recall again that according to our interpretation embryo VI 2 (see Figs. 117-119) is genetically a triple formation.

The first of the questions posed in the introduction to Part
V (page 3), namely, whether the various double formations obtained
in the Schultze experiment from R. fusca eggs can be traced back
ontogenetically to a single basic type, is answered by the above
exposition: The great majority of the different forms indeed
originate genetically from the Schultze duplicitas cruciata. The
possibility of belonging to a basically different type, namely that
of the Spemann duplicitas cruciata exists only in relation to a few
of the embryos we have described.

2. The relationship between mode of gastrulation and the configuration of the resulting double formation.

In Part IV A we described, in the main, three different types
of gastrulation with reference to their external features: 1. ap-
parently normal gastrulation with a circular blastopore, 2. develop-
ment of an initially wide, subsequently narrowing groove, and 3. the
initially narrow groove. It is natural to inquire whether these dif-
ferences in the apparent course of gastrulation can be somehow
brought into relation with the resulting type of double formation.
The general answer to this question is that, on the whole, the various
types of double formation do not depend in any definite way on the
mode of gastrulation. This also seems quite self-evident, if we
bear in mind the fact (Part IV B) that the internal processes con-
nected with the three outwardly so different modes of gastrulation
are in principle the same: In typical cases an archenteric roof
always develops internally in two opposite directions.

In particular, concerning the double formations described
above the following should be briefly noted (see Table VIII). The
same, or approximately the same double formations can develop in the
presence of completely different modes of gastrulation, i.e., in
spite of dissimilar initial development the final stages may largely
conform and, conversely, though the early stages are quite similar,
the final stages may be vastly different. This follows directly from
the above-mentioned table for the typical Schultze duplicitas cruci-
atae, except for their Wetzel form. We see that they occur in the
presence of a narrow groove, a narrow groove in conjunction with an
animal blastopore rudiment, a broad groove, an apparently normal
blastopore, and finally an apparently normal blastopore in conjunc-
tion with an animal blastopore slit. Like Wetzel himself, we could
obtain the Wetzel form where a circular groove was present. We are
not yet in a position to state definitely whether any causal relation-
ships exist. We obtained too few cases, which, moreover, were not

TABLE VIII

To demonstrate what kinds of double formation develop in the presence of outwardly different modes of gastrulation, compiled from data on embryos analyzed in 1925 and 1926. In connection with this table it should be noted that embryos which gastrulated with a narrow groove are relatively underrepresented, since, naturally, for the examination of the individual specimens all the forms of gastrulation had to be selected, even those which occur less frequently. Accordingly, the latter are relatively overrepresented.

Kinds of embryo	Circular groove	Narrow groove	Narrow groove + animal slit	Wide groove	Normal blasto-pore+animal slit	Normal blasto-pore	Total
Typical duplicitas cruciata (Schultze)		14	4	7	3	5	33
Wetzel form of duplicitas cruciata	7	-	-	-	-	-	7
Duplicitas post., "dish-and-lid" embryo	1	-	-	2	-	1	4
Duplicitas post., one head immediately rudimentary	-	1	2	-	3	1	7
Duplicitas ant.-lat., one dorsum rudimentary	-	2	-	1	-	-	3
Duplicitas ant.-lat., displacement of point of intersection	-	6	-	-	-	-	6
Secondary ventral twins	-	-	1	-	1	-	2
Primary ventral twins	-	10	-	-	-	-	10
Single, one head and one dorsum rudimentary	-	4	1	4	-	3	12
Single, Spina bifida	1	13	-	-	-	2	16
Single, Primary	-	7	1	7	-	5	20
Single, Secondary	-	13	-	3	-	-	16

sufficiently well examined. It is noteworthy that in our experiments
they appeared only in the embryos of a single female. It is possible
that amongst the embryos cited in Table I as having died prematurely,
there were other Wetzel duplicitas cruciatae which may have developed
in accordance with a quite different mode of gastrulation. Further-
more, it is apparent that in the case of posterior duplications, in
spite of the fact that only very few occur, almost all above-mentioned
modes of gastrulation are represented; the same applies to single
embryos, except when secondary. To some extent the latter occupy a
special position. We were able to obtain them only after groove
formation. The reason for this, however, lies in the nature of
their development. A normal blastopore, of course, must be excluded
here, since if one is present, the external picture of gastrulation
provides no sure criterion for a decision concerning the secondary
or primary nature of a single embryo. Insofar as the evaluation is
based on outward appearance alone, it may be said that secondary
single embryos depend on the mode of gastrulation to the extent that
they can develop only after the formation of a groove.

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We could obtain only two secondary ventral twins. One of them
displayed a narrow groove, the other an apparently normal blastopore.
Accordingly, there is no dependence on the mode of gastrulation. In
both instances an animal blastopore slit was also present. The
latter, however, has nothing to do with the nature of the resulting
double formation itself. It is found in many other cases too. Its
appearance is probably connected with the properties of the egg ma-
terial. In both cases the eggs were from female VI, from which we
obtained many gastrulae with an animal blastopore slit.

In the case of the remaining forms, namely, primary ventral
twins and anterior and lateral duplications, a certain correlation
seems to exist between the nature of the blastopore formation and
the form of the developing double formation. We obtained ten
primary ventral twins. They all appeared after gastrulation in the
form of a narrow groove. In the case of gastrulation with a broad
groove and an apparently normal blastopore we did not, it is true,
obtain any primary ventral twins among the embryos analyzed; but to
derive from this a definite causal connection between the appearance
of the latter and a narrow blastopore groove seems to us unjustified,
as the numerical data are by no means sufficient. The same applies
to anterior and lateral duplications which we only observed in con-
junction with groove formation. Here, however, it must be added that
in the case of the development of such a double formation from a
cruciata by displacement of the original point of intersection (see
Figs. 86-88), at first glance there would seem to be some connection
with gastrulation with groove formation, since with this modified
form we observed that, essentially, the groove progressed from its

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point of origin in one direction only. Simultaneously it gradually closed, starting from the end of origin. It is a different question, however, whether this kind of groove formation and closure always accompanies the development of the type of modified cruciata concerned. The answer to this question is negative, since we also encountered the same mode of gastrulation with all the embryos that gave a secondary single formation.

The answer to the third question posed in the introduction to Part V must therefore be as follows: in general, in spite of outwardly apparently very different modes of gastrulation, exactly or approximately the same double formations may arise; and conversely, in spite of totally identical gastrulation the most diverse types of double formations and single embryos may result. Only secondary single formations, judging from the outward gastrulation picture, clearly occur only after the formation of a groove. This by no means implies, however, that a closer examination would not reveal the occurrence of secondary single embryos after an apparently normal gastrulation.

3. The relationship between the embryonic rudiments and the original regions of the egg distinguishable before inversion, especially the gray crescent material

In the case of the normal egg, shortly before groove formation three different regions can be distinguished (see Part I, page /325, Fig. 2). Some time after the end of gastrulation the medullary plate appears. According to the older authors, its anterior end definitely does not lie in front of the animal pole, but rather somewhat behind it; and according to the data obtained by Brachet (1923, 1927) its location is even just in front of the animal edge of the gray crescent. From there it extends posteriorwards on the dorsal side beyond the vegetal pole. Thus, in the brown frog, the embryonic anlage (medullary plate, notochord, segmental mesoderm) arises on the dorsal side of the egg, denoted by the gray crescent, the anterioposterior direction of the embryo being oriented in the same sense as the animal-vegetal axis of the egg.

In answer to the question, how the embryonic anlagen of the embryos formed from inverted eggs behave in relation to this normal initial orientation, the reader is referred to the following survey.

Survey of the relationship between the embryonic anlagen
of the embryos described and the position and
orientation of the gray crescent.

I. Out of 35 typical duplicitas cruciatae:

   a) the heads of 15 lie in the region of the gray crescent,
       one to the right, the other to the left of center;
       they are oriented at right angles to the original
       anterioposterior direction. Of the two dorsi, the
       vegetal is normally oriented, the animal oriented in
       exactly the opposite direction (Figs. 51 and 55);

   b) in nine cases both dorsi are oriented at right angles
       to the original anterioposterior direction and lie
       partly within one horn of the gray crescent; of the two
       heads, one is normally oriented in the dorsal direction,
       the other in the ventral direction; more precisely,

       1. in four cases, starting from the middle of the vege-
           tal surface, one is in the central region of the
           gray crescent, the other ventral to the gray cres-
           cent (Figs. 56 and 60);

       2. in five cases, starting from the right or the left
           edge, in one horn of the gray crescent (Fig. 67);

   c) the heads of 10 are oblique with respect to the original
       anterioposterior direction, one dorsal to left or right
       in the gray crescent, the other in the left or the
       right horn of the gray crescent. The dorsi are also
       obliquely oriented, one vegetal, the other animal (Figs.
       61 and 62);

   d) the rudiments of 1 are rotated through exactly 180
       degrees relative to those illustrated in Figs. 61 and
       62; the heads are ventral, one possibly still within the
       right horn of the gray crescent, the other definitely
       not (this embryo is not more closely described).

II. Out of 8 posterior duplications the well developed head

is

   a) in 3 cases perpendicular to the normal direction, or
       more precisely

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      1. in 1 case to the right in the gray crescent (Figs. 6 and 78),

      2. in 2 cases to the left in the gray crescent (Fig. 80), (of the two dorsi, the vegetal is normally oriented, the animal rotated through 180 degrees),

b) in 3 cases dorsal in the gray crescent, perfectly normally oriented, or only slightly oblique (Fig. 77)

c) in 2 cases inverted, slightly oblique in the right horn of the gray crescent; the dorsi are also correspondingly oblique (Fig. 76).

III. Out of 7 anterior or lateral duplications

a) in 4 cases the embryonic anlagen are vegetal, or more precisely:

      1. In 2 cases the heads lie to right and left in the gray crescent

         α) both approximately normally oriented (lateralis, Fig. 86),

         β) or approximately at right angles thereto (anterior, Figs. 83 and 84), (the dorsi are normally oriented),

      2. in 1 case (lateralis), exactly the reverse of the orientation of a normal embryo, the heads being located ventrally (the embryo is not described more closely),

      3. in 1 case (lateralis), rotated to the left through 90 degrees relative to the original orientation (likewise not described);

b) in 3 cases (anteriores), the embryonic anlagen are animal, the heads dorsal; almost completely the reverse of normal, the dorsi directed exactly opposite to normal (Fig. 87).

IV. Out of 8 ventral twins

a) in 1 case one anlage is normally oriented lying dorsally

on the vegetal surface, the other is also vegetal, but ventral and inverted (Fig. 89);

b) in 1 case both anlagen are on the vegetal surface rotated through 90 degrees to the normal, one to the left, the other to the right (not more closely described);

c) in 6 cases both anlagen are on the edge, one dorsal, i.e., rotated through 90 degrees with respect to normal, the other:

  1. in 2 cases on the left, rotated through 180 degrees (Fig. 92),

  2. in 3 cases on the right, also rotated through 180 degrees (Fig. 93),

  3. in 1 case ventral, rotated through 90 degrees with respect to the normal (Fig. 91).

V. Out of 48 single formations the embryos are:

  a) in 20 cases normal, with the head dorsal in the gray crescent (Fig. 110);

  b) in 7 cases rotated through 180 degrees, the head ventral on the vegetal surface (Figs. 16 and 106);

  c) in 6 cases rotated through 90 degrees, or more precisely

  1. in 3 cases with the head in the right horn of the gray crescent (Figs. 10 and 101),

  2. in 3 cases in the left horn (not described);

  d) in 12 cases on the edge, or more precisely

  1. in 9 cases:

    α) 7 with the head in the right horn of the gray crescent (Fig. 100),

    β) 2 in the left horn (not described),

    2.   in 2 cases ventral, with the head on the left (Fig. 107),

    3.   in 1 case on the left, with the head dorsal (Fig. 111);

e)   in 3 cases on the animal side, or more precisely

    1.   in 1 case normally oriented, head ventral (not described),

    2.   in 1 case rotated through 180 degrees, head dorsal (Fig. 112),

    3.   in 1 case rotated through 90 degrees, head on the right (not described).

CONCLUSIONS

The survey above permits three conclusions. First of all, any part of the surface of an inverted embryo (except for the light white areas) can become any part of an embryonic anlage. If all the embryos examined are drawn in vegetal view one above the other with the gray crescents superimposed and the corresponding embryonic anlagen added, it will be found that all the regions of the surface are covered with embryonic anlagen. But, secondly, this also implies that in the case of inverted embryos embryonic anlagen not only appear on the dorsal half of the egg, but can develop partly or, indeed, only on the ventral side; and this ventral anlage may even be better developed than the dorsal one. This follows, for instance, from the fact that in Group Ib of the above survey the dorsal and ventral heads were equally well developed on 5 occasions; in one case the ventral head was even better developed than the dorsal head. The significance of these facts is not diminished by the further observation that in 3 embryos the dorsal head was better developed. Further corroboration is provided by the fact that of the two embryos described where only one half was differentiated in one case (Fig. 104) it was the ventral half that developed and in the other case (Fig. 105) the dorsal half. Thirdly, with regard to the direction in which their anterior ends develop, the embryonic anlagen of inverted embryos bear no constant relation to the normal initial structure of the egg before inversion. Thus, the fourth question (see Introduction to Part V) is answered.

Of particular interest to us is the question of the exact location of the frontal groove in the individual embryos, that is, whether or not it divided the embryo in such a way that the ventral half also received part of the middle region of the gray crescent. In this connection the following points may be made:

1. In a considerable number of embryos the first anlage of the frontal groove appeared almost exactly at the original vegetal boundary of the central region of the gray crescent, that is to say, in the normal location (for example, Fig. 70a, 76a). In such cases the whole central region of the gray crescent arrives in the dorsal half of the embryo. In spite of this, not only the dorsal but also the ventral half was able to develop an embryonic anlage.

2. We have closely examined our drawings to see if the first anlage of a frontal groove can also develop above the vegetal boundary of the central region of the gray crescent, that is to say, in the central region itself. In such cases part of the gray crescent would fall to the ventral half of the embryo. Originally (1925, page 144) we believed that this would have to be the case, if the ventral half were to form an embryonic anlage. But definite examples of the blastopore anlage developing at the location in question can not be found. Moreover, in view of the nature of yolk displacement and the facts upon which we have based our concept of the causality, of double gastrulation, it is improbable that the blastopore anlage if it runs frontally, could occur dorsally displaced in the manner described.

3. Among the more closely described of the embryos examined the first rudiment of a frontal narrow groove is not infrequently formed quite definitely below the vegetal boundary of the central region of the gray crescent, that is, within the limits of the original vegetal field (see, for example, Fig. 57a, 98a). If an initially broad, frontally or approximately frontally directed groove develops from two originally widely separated anlagen, the ventral anlage always lies within the limits of the original vegetal field (see, for example, Fig. 20 and 28), in fact, even almost on the ventral edge (see Fig. 21). Moreover, the first blastopore anlage is frequently situated in the vegetal field, even when not a groove, but an apparently normal blastopore develops (see, for example, Fig. 74a). In all these cases, the ventral half of the embryo has certainly received nothing of the central region of the gray crescent, and yet was often able to develop an embryonic anlage. Such cases are not infrequently among the embryos described, only because we purposely selected them as such. In reality they only form exceptions. We formerly believed (1925, page 143) that in the

case of inverted embryos blastopore anlagen could only appear in the area of the gray crescent. This view can no longer be maintained. The first blastopore anlage can appear, though rarely, outside the region of the gray crescent, whatever the mode of gastrulation.

Now we can also answer the fifth of the questions raised in the introduction to Part V. As a rule, the first blastopore anlage appears at the vegetal edge of the central region of the gray crescent and, in the event of a median or oblique location of the groove even in this region itself. As was often emphasized in discussing the individual embryos, the involution is most abundant at the site of the first rudiment of the groove, and therefore (see next section) the anterior ends (heads) of the double formation also appear here. This explains why, in most cases, the head rudiments were to be found in the region of the original gray crescent; but, as follows from the above survey, this region is not the only place where head rudiments may appear.

4. **Explanation of the origin of double formations after double gastrulation.**

The findings relating to the causes of double gastrulation are presented in detail in Part IV C 4. As a first consequence of gastrulation two blastopore margins appear, oriented with their involution lips opposite one another. Secondly, we get two archenteric roofs which develop in opposite directions. How two embryonic anlagen result therefrom can be explained in two different ways. We can start from Marx's (1925) contention that in Triton the involuted archenteric roof, which itself becomes the notochord and somites, determines the development of the overlying ectoderm as the medullary plate. We have already applied this concept in describing the development of the individual double formations, and have illustrated it schematically. Alternatively, the two blastopore margins might exert a dynamic influence, in Goerttler's (1927) sense, upon the material in front of them, without either the involution or archenteric roofs or its effect on the overlying ectoderm playing a direct part. The result could again be the formation of two embryonic anlagen.

a) The first of these two theories will be examined in more detail. Thus, according to Marx and the Spemann school, in general, an archenteric roof determines development of the overlying ectoderm as a medullary plate. Since in the case of inverted embryos two archenteric roofs are formed, they are also characterized by two medullary plates, two embryonic anlagen. The directions in which involution of the archenteric roofs occurs determine the directions in which the anterior ends of the two individual parts face and the

part of each archenteric roof that has turned inward the farthest,
determines the development of the overlying ectoderm (and perhaps
partly the ectoderm in front of it) as the anterior end of the
medullary plate. The development of a straight, initially broad
from the outset, or, narrow groove is associated with two parallel
blastopore margins directed towards each other. Each of these
corresponds to the entire margin of a normal circular blastopore.
In describing the development of double formations, we have several
times pointed out that a fairly intense involution normally occurs
at only one point along the groove, namely, at the point where the
groove was first laid down. This point on each blastopore lip, where
the archenteric roof is turned inward the farthest, must be equated
with the dorsal margin of a normal blastopore. As we have also
frequently stressed, it usually, but not always, lies in the region
of the original gray crescent. Moreover, the two major points of
involution are located opposite one another on the groove. Thus it
is understandable that both the primary single anterior ends of the
double formations should originate in one point on the groove (the
point of intersection of the cruciata), generally in the region of
the gray crescent. The involution of surface material also occurs
around the remaining parts of each blastopore lip, to the right
and left of the major point of involution; this, however, occurs to
a lesser degree. The parts of the archenteric roof thus formed on
each side of the groove likewise determine the development of the
overlying ectoderm as medullary plate, namely, as the right and
left neural folds of each of the two individual parts. The two
folds must, therefore, run to right and left along the groove from
the anterior end of each individual part, i.e., remain separated
from one another as in a spina bifida. Since in the meantime the
groove closes, however, the right neural fold of one individual
part grows into the left neural fold of the other, and vice versa.
In this way, the secondary single posterior ends of the cruciata
are formed. The theory developed above is illustrated in Figure
121 II.

This theory also explains the genesis of the numerous modified
forms of double formations and single embryos. Thus, for example,
the primary anterior ends of the cruciata are shorter or longer
depending on the intensity of the involution of the archenteric roof

in the direction of the longest arrows (s and s^1 in Fig. 121 IIa).
If the involution does not proceed in exactly opposite directions,

but rather so that the arrows s and s^1 enclose an angle of less
than 180°, the primary anterior ends of the cruciata are likewise
oriented. If, for some reason, involution does not take place
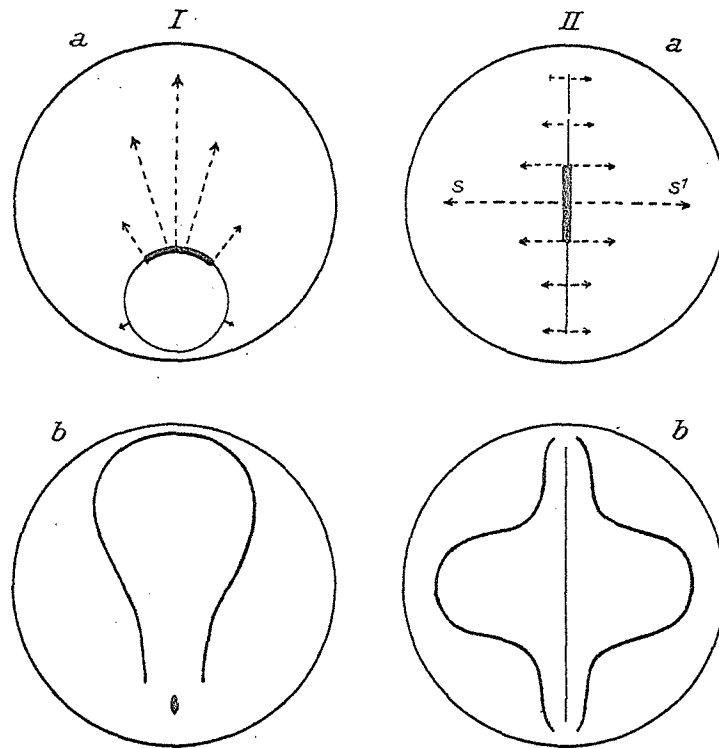
Fig. 121.

Schematic. I. Normal embryo: a) dorsal blastopore lip,
thick line, rest of blastopore lip, thin line; the ex-
tent of the involution of the archenteric roof around the
various parts of the blastopore lip is indicated by the
length of the arrows. b) Blastopore closed; shape of
the medullary plate. II. Embryo from an inverted egg
with narrow (median) groove. a) the portion of each
of the two groove lips corresponding to the dorsal
blastopore lip is indicated by the thick line, the
other parts by the thin line. The arrows have the same
significance as in Ia. b) Groove is closed, shape of
medullary plate of duplicitas cruciata.

around one lip of the groove, the formation of an embryonic anlage
is also suppressed on that side. Fig. 121 IIa also suggests how a
duplicitas cruciata can follow the formation of an apparently normal
circular blastopore, to the extent that the major involution occurs
at two opposite points along the lip. It is not necessary to
work out this idea in detail for the different forms of the
Schultze double formations, since this has already been done in
Part V. We shall simply then add that the variable involution
of material on either side of the arrows s and s^1 could also serve
to explain how, as Fräulein Wittmann will show, the notochords and
somites in the secondary posterior ends of the developed cruciata,
may evolve with varying degrees of success.

This entire concept, however, is hypothetical. In the first
place, in the case of the Salientia it has not yet been definitely
established that the archenteric roof is formed only by involution.
Secondly, and particularly important, it is not known whether the
medullary plate is irreversibly determined before or only after the
beginning of gastrulation. If the first alternative, which is
perhaps the more likely, is the right one, we would have to seek a
different explanation for the origin of double formations.

b) This second possible explanation is connected with
Goerttler's (1927) assumption that dynamic influences emanate from
the dorsal blastopore lip, so that the entire surface of the embryo
becomes a "field," in which an energy gradient exists, radiating from
the energy maximum in the dorsal blastopore lip. According to our
findings, the site of the first blastopore anlage is determined by
the influence of white yolk material on matter capable of involution,
as a rule the material of the gray crescent. The result of the
displacement of the yolk in inverted embryos is that a groove is
formed, i.e., two dorsal blastopore lips, with the margins directed
towards each other. Thus, there are two points at which an energy
maximum exists and from which energy gradients run in opposite di-
rections. The anterior and lateral boundaries of the medullary
plate may be determined by a certain stage of this energy drop.
It is conceivable that in the normal embryo an energy gradient
emanates not only from the dorsal but also from the lateral blasto-
pore lips though in the latter case it will be shorter, since less
energy is localized in the lateral lips. If, in accordance with
these assumptions, we now indicate the energy gradient on the surface
of a normal embryo up to the stage that determines the outer limits
of the medullary plate by means of arrows of different length, the
line formed by the tips of these arrows will approximately correspond
to the outer limit of the presumptive medullary plate of the amphibian
embryo (Fig. 122 I). If we do the same for an embryo formed from an

inverted egg and having two blastopore lips, the arrows of varying
length will indicate the boundaries of the two medullary plates.
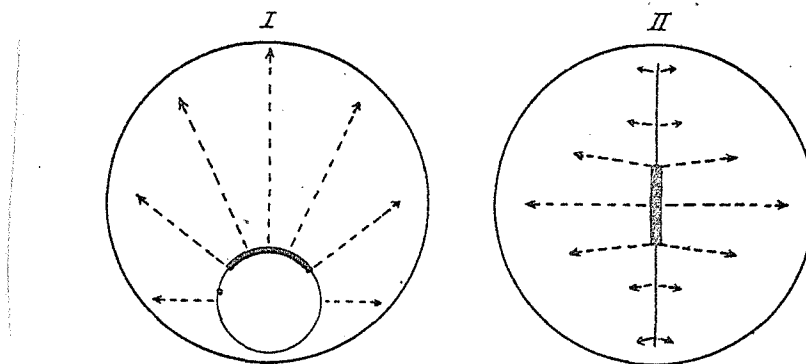
Fig. 122.

Schematic representation of the hypothesis of a
dynamic influence emanating from the blastopore
lip and a consequent energy gradient. The lat-
ter is indicated by arrows up to the stage that
determines outer boundaries of the medullary
plate. I. Normal embryo. II. Embryo from an
inverted egg. The dorsal blastopore lip and the
remaining parts of the blastopore lip are indi-
cated as in Figure 121.

Naturally, these arguements are just as hypothetical as the first   /138
assumption (see a above). However, they offer the possibility of
explaining the origin of Schultze double formations without it being
necessary to assume the determination of the medullary plate primarily
as a result of the involution of the archenteric roof.
          In principle, therefore, it is possible to explain the origin

of Schultze double formations in terms of the special character-
istics of the gastrulation of inverted embryos. Thus, we have
also answered the second of the questions raised (introduction
to Part V).

PART VI: GENERAL

1.   Comparison of the Schultze Inversion Experiment
With Other Experiments in the Young
Amphibian Embryo

In the Schultze inversion experiment, parts of an egg which
has just begun cleavage, or parts of the first two to eight blasto-
meres, shift position according to their various specific weight.
This displacement concerns only the yolk material and partly also
the pigmented cells, while the substance of the gray crescent remains
in place (cf. part IV A 7).   To account for these findings we as-
sumed that in the egg of R. fusca, an outermost layer of ectoplasm
is present which is not pigmented and no longer recognizable in the
preparation, and that the gray crescent represents a specific,
localized differentiation in this layer (cf. part II 3).   In ac-
cordance with the interpretation briefly recapitulated given above,   /139
the Schultze inversion experiment thus involves essentially the
rearrangement of the material in the interior of the egg and be-
neath the outer layer of the egg plasma, so that the regions of this
outer layer are placed under new conditions.
Fundamentally, the same result is achieved if one does not
rotate the vegetal pole of the egg completely to the dorsal side.
Then the white yolk sinks, as Born (1885) demonstrated, only towards
one side;   under these conditions too, the unaltered outer layer of
the egg, which has remained in place, assumes other positional re-
lationships to the yolk material.   This experiment has been recently
repeated on the undivided egg by Weigmann (1926, 1927).   We shall
return to the results of this later.   Here, we shall only note that
he did not follow the development of the embryo up to the appearance
of the embryonic anlage, and that it is therefore unresolved, whether
double formations can arise in such an experiment.   According to
our theories of the causality of gastrulation, this should be the
case.   For if, in the experiment cited, the white yolk sinks to-
ward the bottom along the "flow meridian", then its upper edge not
only borders on the material of the gray crescent, but also on
other "dark" material.   Consequently, the conditions under which a
blastopore anlage occurs must also be present elsewhere.   It is not
very probable that these conditions are then realized if one ro-
tates an undivided egg so that the vegetal pole is completely up-
ward.   We have already reported on such experiments (1925, p. /139)
and at that time, we obtained only one double formation.   Since
numerous repetitions of the experiment were without success, one

might have thought that the experimental conditions were peculiar in that case: Perhaps that egg had not been completely inverted upward with the vegetal pole, contrary to our intentions. Inversion experiments on the undivided egg, conducted in different manner, ought to be suitable as a test to see whether our theory of the causality of the blastopore anlage is valid.

The shifting of the specificially differing heavy materials in the amphibian egg can be achieved not only by the gravitational force but also by the centrifugal force. In such a case, the question is whether the outer layer of the plasma, in particular that portion which forms the gray crescent, is not also shifted. Centrifuging experiments have been frequently conducted on amphibian eggs, first by O. Hertwig (1897 and later); for the most part, only the experiments by G. Wetzel (1904) are considered here. He centrifuged frog eggs in the unfertilized state, in such a manner, that the animal side was centrifugally oriented; the white yolk was thus, as in the Schultze experiment, and certainly to an even higher degree, shifted into the animal egg half. The eggs could be fertilized and then the animal side, corresponding to this yolk displacement, divided more slowly than did the vegetal side. Despite this, the blastopore evolved on the original vegetal side at the place where it would also have appeared under normal conditions. One might gather from that, that the position of the blastopore anlage has already been formed prior to fertilization, and indeed, in a material which was not displaced by the centrifugal force. That fully coincides with our results concerning the unshifting nature of the gray crescent when under gravitational force. Wetzel did not obtain double formations in these experiments. We would like to express our doubts as to whether Wetzel's results concerning the site of appearance of the dorsal blastopore lip in centrifuged eggs are valid, however. It may also have occurred, as in our experiments, that the blastopore could be laid down as a rule at the originally determined spot, but in many cases, it could also be laid down at other spots if this is determined by the altered arrangement of the white yolk (cf. the influence of the bright-white areas in our experiments).

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Recently, Bagini (1923) has described double formations which developed from centrifuged eggs of _Bufo vulgaris_. The eggs were in part centrifuged at the time of the first cleavage, and in part only at the blastula stage. The author has nothing to state concerning the behavior of the eggs in the first case; she accounts for the double formations obtained from the blastulae by assuming the displacement of blastomeres or parts of the embryo which had already differentiated. Neither the developmental conditions produced in the experiment nor the results according to Bagini's very short report can be judged more precisely.

The Mangold experiment (1920, cf. also Mangold and Seidel, 1927) in which two entire eggs in the two-cell stage are fused in crossed position or the blastomeres of a four-cell stage egg are displaced, may perhaps be carried out in such a manner that the organization center is brought into an abnormal positional relationship to the white vegetal field and thus, alterations of the developmental conditions similar to those of the Schultze experiment are created. For that purpose, it would be necessary, however, to conduct the experiment in such a manner that the region of the organization center would be visible on the eggs.

Spemann (1901-1903) and Hey (1911) have obtained cruciatae by incomplete constriction of Triton eggs at the two-cell stage. Their genesis has not been more precisely described yet, but in our opinion, they belong to the Schultze type. It can be readily imagined that a double gastrulation completely similar to that of the inversion experiment is produced by such a constriction. Frälein Wittmann will discuss this in more detail.

Of the other experiments on the egg and in the early cleavage stages, the complete separation of the first two blastomeres (Spemann 1901-1903) offers points of comparison to the Schultze experiment which has already been mentioned in part I (p. /319) and to which we shall return again. In Spemann's basic experiment (1918 and later), as well as in numerous other supporting experiments, developmental conditions completely similar to those of the inversion experiment are created, i.e., conditions under which organizer material is brought into an abnormal positional relationship to the other parts of the embryo. Here too, blastopore formation occurs in an abnormal site, double formations evolve, etc., and these experiments can also provide information on many of the questions which crop up in the Schultze experiment. But since the Spemann investigations relate to later developmental stages, they will not be examined in greater detail here.

2. Comparison of the Results With Other New Findings on the Blastocoel and the Gastrulation of Anura in Normal Development

In part IV, in the representation of the gastrulation of inverted embryos, we proceeded from Goette's old conception (1875), according to which the initially narrow gastrulation slit widens in normal development and, as a consequence, the blastocoel is completely suppressed by the white yolk which descends into it. But other authors, primarily O. Schultze (1888), have shown that sometimes a part of the blastocoel remains and is unified with

the archenteron when the partition wall of endoderm cells which
initially is present in between them becomes thin and collapses.
According to Meek's investigations (1923), of R. fusca the blasto-
coel was retained in all cases and regularly fused with the narrow
archenteron, providing the anterior portion of the gut cavity.

As a control for the material from which we obtained double
formations, we have fixed and sectioned several normal embryos at
various stages of gastrulation. From the preparations, we can con-
firm that in these normal embryos, at least a portion of the blast-
ocoel is not suppressed by the descending yolk and, judging from
the thin wall of separation, it would later have united with the
archenteron cavity. This had already very much widened at a time
when its connection with the blastocoel had not yet occurred, and
the widening of the initially very narrow gastrulation slit in the
normal embryos examined is very much greater than in the inverted
embryos: in the latter it almost always remains very narrow and
widens only rarely into a larger cavity. On the basis of this dif-
ference between normal and inverted embryos, we may assume that in
normal development, the blastocoel actually becomes narrower as a
consequence of the descent of the white yolk and the widening of
the archenteron. This process is mainly absent during the gastru-
lation of inverted embryos, since in the embryos the white yolk has
descended a priori into the animal egg-half. But it is also pos-
sible that different fusca material may behave somewhat differently
depending on the extent to which the blastocoel is narrowed during
normal gastrulation and that differences in the arrangement and
quantity of yolk play a role in this.

We will not go into the questions of comparative developmental
history which have been discussed in connection with Meek's find-
ings and in connection with the same observations by Rao and Ramanna
(1925), mentioned below, by the authors named as well as by
Goodrich (1925). The respective presence, absence or number of
blastocoels has no significant effect on the result of the Schultze
inversion experiment since only the spatial relationships of the
cavities are concerned in the double formations.

Rao and Ramanna (1925) describe the same process of unifica-
tion between the blastocoel and the archenteron in Engystomatids
(Cacopus systoma, Microhyla ornata and Callula variegata) as well
as in Rana tigrina and Bufo melanostictus. In addition, however,
the normal development of the Engystomatids cited displays several
highly striking and remarkable resemblances to the development of
inverted eggs of Rana fusca.

First, two blastocoels are formed in the Engystomatids: the
primary, larger one lies more toward the animal side, the smaller,
secondary one which appears later, develops in the vegetal part of
the yolk. Both of them subsequently fuse with the archenteron. We

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also found two blastocoels in a small number of cases in inverted
fusca eggs.

Second, the blastopore in the Engystomatids forms from two
initially separated anlagen; a ventral blastopore lip develops
simultaneously with the dorsal blastopore lip and is completely
separated from it at first; beneath both of them lies a gastrula-
tion slit which leads into the interior of the egg. That corres-
ponds exactly to the formation of a blastopore from two anlagen as
has been described above for several rotated fusca eggs(cf. for
example part IV, Figs. 21 and 22). In such cases, the double
blastopore anlage leads to an "initially broad groove" (cf. part IV,
p. /368); we consider it possible however, for a "seemingly normal",
that is, roundish blastopore, to develop from two initially separated
anlagen. According to Rao and Ramanna, the latter proves correct
for the normal development of the Engystomatids: The dorsal and
the ventral blastopore lips, which, as stated, are initially
separated from one another, unite at their ends to form a ring-shaped
blastopore edge. We have now traced double gastrulation, that is,
the formation of two archenterons from opposite directions, as well
as the origin of the double formations in general, back to the
fact that the two blastopore lips arose with their turned edges
oriented toward each other. As stated, this normally occurs in
the Engystomatids, for among them, the dorsal and the ventral
blastopore lips are oriented with their turned edges toward each
other so that two archenteric roofs are invaginated from opposite
sides. The archenteron cavities beneath them are also of equally
extensive development. If our explanation of the origin of double
formations were to be extended to the Engystomatids, it would re-
quire that these yield double formations in the normal fashion,
which of course is not true. The different results of development
in inverted embryos and in the Engystomatids are not explainable,
despite very similar behavior of their blastopore anlagen. The
eggs of all the amphibians which we used for the inversion experi-
ments develop slowly. In Rana fusca,the blastopore is closed after
about three days or more, depending on the temperature, during
normal development; in the Engystomatids, the closure may al-
ready have occurred after twelve hours, so that Rao and Ramanna
speak of a phenomenal acceleration of development. A supposition
strongly suggested by this extraordinary difference in the speed
of development is that the determination of the organ anlagen has
been definitely completed very early, that is, already prior to
gastrulation, and that consequently, the archenteric roof that is
initially invaginated from two opposite directions cannot exert
any further determining influence on the ectoderm. It would be
worth a test to see whether the Engystomatid embryos have, as we

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presume, lost the capacity for regulation already prior to gastrula-
tion. In the case of the Engystomatids also the initially separate
appearance of two parts of the blastopore edge cannot be traced
back to the presence of two blastocoels since this is contradicted,
by their positions, as described above, and by the tardy appearance
of the secondary blastocoel.

The third similarity between the development of the
Engystomatids and the inverted eggs of Rana fusca consists of the
fact that, according to Rao and Ramanna, in the former group, the
archenteric roof partly arises through invagination and partly is
differentiated through division on the spot, and consequently, by
means of a process which we must also take into consideration in the
case of the inverted embryos (cf. part IV B 2 d). We have,however,
explained that the actual appearance of an archenteric roof for-
mation by means of division cannot be demonstrated in the prepara-
tions and, likewise, the diagrams presented by Rao and Ramanna
cannot convince us that such a process is realized in the Engy-
stomatids either. According to Brachet (1927), in normally develop-
ing Rana fusca the notochord and somites are formed from involuted
material, namely, from that material from the lower (more vegetal)
part of the gray crescent, while the rest of the mesoderm develops
by differentiation on the spot.

3. Lability of the Organ Rudiments During the First Cleavage Stages

The boundaries of the presumptive organ rudiments have been
determined in detail with great certainty in the Urodela embryo by
Vogt (1925, 1926 and earlier) and by Goerttler (1925), by means of
local vital dyes. More recently, Brachet (1923) has attempted to
elucidate these relationships in the Anura by means of pricking
experiments and has recently commented extensively (1927) on his
results and their relationship to the results obtained in
Urodela. According to Brachet, the presumptive medullary plate,
notochord and dorsal segments are present in the egg and in the
blastula of R. fusca, in addition to the presumptive epidermis (=
animal field) and the presumptive endoderm (= vegetal field); the
anlagen of the first three of these lie together in the gray
crescent. In this area, we should differentiate a part situated
more toward the animal side, the presumptive medullary plate,
and a part situated more toward the vegetal side, the presumptive
notochord and dorsal segments. Accordingly, the region which
contains the anlagen of the three organs named has in the meridional
direction, a much smaller breadth in the Anura than in the Urodela.
However, according to Brachet, there are still two things to be

added. First, the gray crescent was presumably of greater breadth originally and narrowed after fertilization. Second, the boundaries of the presumptive anlagen of the medullary plate, notochord and somites cannot be determined precisely since the gray crescent corresponding to them can be different in clarity and breadth; in many cases, even the material which borders on the visible gray crescent can be considered as belonging to the region of those organ rudiments. Without wishing to detract from the value of the defect experiments, one must nevertheless admit, that the results obtained through the use of this method are not so free of objection as those achieved by Vogt and Goerttler. Still one may, at least provisionally, take the following statement as a basis for their consideration: Our investigations of the inverted embryos did not, in any case, demonstrate any facts that contradict Brachet's presentation.

Brachet (1906) has arrived at the opinion, through pricking experiments conducted at different times after fertilization, that the egg of R. fusca is capable of complete regulation only within the first 45 minutes after fertilization; 1 - 1/4 hours later, it has almost completely lost the capacity for regulation, for by then defects in the egg will have resulted in corresponding defects in the embryo. By then also the gray crescent is nearly formed and the presumptive anlagen of the medullary plate, the notochord and the somites are first localized of all (however, they naturally undergo positional changes later) and then irrevocably determined. However, Brachet (1927) also recognized a certain capacity for regulation in the fusca egg at a somewhat later stage: MacClendon (1910) obtained from an half-blastomere of a Chorophilus egg (Anura) a complete embryo, following the removal of the sister cell; according to the Schultze experiment two embryonic anlagen develop from one egg and Morgan (1895) has obtained a complete embryo from 1/2 a blastomere of an inverted egg. At the completed two-celled stage, according to Brachet, however, the capacity for regulation has been lost, because by them the inversion experiment no longer gives rise to double formations.

This deduction by Brachet is no longer tenable, for we have even obtained double formations in R. fusca at the four- and eight-celled stage. This is shown by the survey of the cleavage stages (Table IX), in which we have obtained double formations from inverted eggs of R. fusca. Thus, the prospective significance of the embryonic regions has not been irrevocably fixed, at least until the eight-celled stage. Tonkoff (1900) has obtained such formations in Triton at the four-celled stage, which does not preclude a longer maintenance of the capacity for regulation in Brachet's Urodela.

We now recall the conclusion that the original gray

TABLE IX

Results of inversion experiments with Rana fusca at different
cleavage stages. Compiled after several experiments in 1925.
The questionable double formations are disregarded as are
dead or unanalyzed embryos, which, according to our current
experience, almost all have been double formations, just as
we are certain that most of the single embryos would have been
recognized today as genetic double formations

Cleavage stage at which the egg was pressed and inverted	Number of normal single embryos	Number of double formations
Second cleavage at the beginning	34	13
Second cleavage encompassing half the egg	40	4
Second cleavage almost or completely finished	76	5
Third cleavage at the beginning	8	4
Third cleavage just finished	6	1
16 celled-stage	35	0

crescent has to also retain its position and configuration the in-
verted egg. But the organ rudiments which become visible externally,
that is, the two medullary plates of the double formation, doubtless
do not coincide with the position and configuration of this region,
even taking into consideration their change in position during de-
velopment. It follows from this that at the stage, in which the in-
version experiment still gives positive results, the determination of
the embryonic regions has not even progressed so far that only the
material of the gray crescent could form the medullary plate (as well
as notochord and dorsal segments) and it is even less likely that all
the individual parts of these organs could have already been con-
clusively determined. The contradiction between the results of the

inversion and the defect experiments indicates, as in many other
cases, that the latter is not suitable for fixing the time of irrevo-
cable determination of organ rudiments.

Our experiments can provide no information as to when this
determination actually becomes irrevocable in the egg of R. fusca.   /147
It is not unlikely that in the Anura, as Brachet thinks, the capacity
for regulation of the embryo is lost significantly earlier than in
the Urodela.

4.   Lability of orientation organization during
the first cleavage stages

After the formation of the gray crescent, the R. fusca egg
possesses a bilaterally symmetric organization that is externally
recognizable. First, a heteropolar principal axis is present which
connects the animal pole with the vegetal; second, the middle of the
gray crescent delineates the dorsal side, by which the similarly
heteropolar dorsoventral axis is indicated; third, in this way, the
median parts can then be differentiated from the lateral (right and
left), and the plane determined by both axes is the plane of sym-
metry. Our experiments tell us nothing about the origin of this bi-
lateralness in the egg. The bilaterally symmetric organization of
the egg is normally carried over into the embryo. But since the lat-
ter possesses an asymmetric Situs viscerum, its axis extending from
right to left is also heteropolar, so that a bilaterally asymmetric
organization comes about. Now the question is whether this organi-
zation of orientation has been irrevocably determined at the stages
during which the inversion experiment gives positive results.

a)   The heteropolar dorsoventral axis is actually fixed to
a high degree. For, as a rule, the blastopore appears, as was des-
cribed in detail in Part IV A 7, in approximately the same spot
where it is normally laid down, namely, in the middle portion of the
gray crescent or a little to one side. Weigmann came to the same
conclusion (1926, 1927) in his investigations on the influence of
the oblique position of constraint on the position of the blastopore
anlage. We attribute the maintenance of the original dorsoventral
axis to the fact that the material of the gray crescent is not af-
fected by the displacements of the yolk material.

In rare instances (to which we devote much attention in this
study solely because they are exceptions from the rule) the blasto-
pore is laid down outside the region of the gray crescent. If the
site of the first blastopore anlage is said to denote the dorsal
side of the incipient gastrula, as is normally the case, the position

of the dorsoventral axis can in fact be altered by the inversion experiment.

b) The position of the anterioposterior line of orientation
is very easily altered. When the groove occupies a median position,
one of the two individuals has its head oriented toward the right and
the other toward the left side of the original organizational pattern
in the egg (cf. Fig. 55); in this case the anterioposterior lines of
orientation of both embryonic anlagen are rotated through 90° to the
original orientation. When the groove position is frontal, the
dorsal individual lies in the anterioposterior line of orientation
which originally occurred in the egg, while in the ventral individual,
this line has been rotated through 180° (cf. Fig. 60). When the
groove is situated obliquely, the direction toward which the anterior
ends of both embryonic anlagen are pointed deviates at a correspond-
ing angle from the original line of anterioposterior orientation (cf.
Fig. 62). The very same result -- the alterability of the anterio-
posterior line of orientation -- can also be drawn from Weigmann's
experiments on eggs in an oblique position of constraint.

Particularly in the case of the gray crescent, the anterio-
posterior line of orientation is not irrevocably preformed. For if
the anterioposterior lines of orientation for both individuals at
median or oblique position of groove are rotated through 90°, the
anterioposterior line of orientation for the parts of the gray
crescent corresponding to the two individuals must also be rotated.
This is especially evident because, in these cases, the anterior ends
of the individuals, by which the anterioposterior polarity can be
recognized, lie in the vicinity of the original gray crescent (cf.
Figs. 55 and 62). Rotation of the anterioposterior polarity through
180° would occur if a frontal groove would transect the middle region
of the gray crescent and an embryonic anlage would develop from it
on the larger half extending toward the ventral side. We did not
find such cases for certain (cf. p /133/), however. If one also
judges the anterioposterior line of orientation from the standpoint
of the secondary posterior ends of the cruciata, which is justified,
in several embryos the anterioposterior line of orientation is cer-
tainly rotated through 180° in the vicinity of the gray crescent.
For example, in embryo VI 5 (Figs. 14 and 66), this region formed a
dorsum rather than the two heads; the original anterioposterior line
of orientation of the gray crescent is directly opposed to that of
this dorsum.

c) As a rule therefore, the dorsoventral line of orienta-
tion is maintained but the anterioposterior line is not. Since the
median plane is determined by both lines of orientation, it must very
frequently be rotated against that of the original median plane. Ac-
cording to Weigmann, in the eggs maintained in an oblique position

of constraint the meridian which happens to be perpendicular and therefore passes through the mass of white yolk underneath, becomes the median; for in such eggs the dorsal blastopore lip forms along the boundary between the descended white yolk and the material of the remaining gray crescent. Similarly, the median plane of the two individuals in the Schultze experiment can be rotated through any angle with respect to the original one, as a glance at Figs. 55, 60 and 62 shows. That this median plane is at least initially common to both individuals in the double formation is explained by the fact that, at least in the typical cases, they are laid down in opposite directions from the groove.

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Thus, from the preceding summary, it follows that the principal lines of orientation in the organization of the egg are not initially unalterable, as Vogt (1926) recently appears to have assumed. They are, however, uniquely determined in the normal egg by differentiation of the gray crescent as well as by the arrangement of the remaining egg material. If the positional relationship between the yolk material and the region of the gray crescent is altered however, other principal lines of orientation are formed. Of course, this result is valid only for the first few cleavage stages, during which the Schultze double formations can still be obtained. According to Spemann (1927) and Goerttler (1927), a much more firmly determined structure of orientation is acquired by the entire embryo and, in particular, by the organization center, during the blastula and early gastrula stages. The degree of evolution of this structure thus increases during development.

Naturally, we were also interested in the question of whether the displacement of the yolk material exerted an influence on the evolution of an asymmetric Situs viscerum. The external examination of the double formations which is reported here can provide no information on this question; Fräulein Wittmann will report on the results of the sectional examination of developed embryos.

5. Quantitative differences in the outer layer of the egg

Normally, the dorsal blastopore lip is laid down obliquely to the dorsal midline, at the boundary between the gray crescent and the white vegetal field. In the inverted egg, it develops at approximately the same place as a rule, that is, in the region of the original gray crescent and very frequently, on the edge of one of the bright-white regions visible at the surface as well. Sometimes, the first blastopore anlage only bears a relationship to such a bright-white spot without, at the same time, belonging to the region of the original gray crescent. There were cases in which a sort of blastopore

even formed on the animal surface in relation to white yolk which had   /150
descended at that point on the surface.

      Normal behavior, as well as that of the inverted embryos, can
be summarized in the following sentence: Any part of the surface
layer which is not too laden with white yolk can display the ability
to form a dorsal blastopore lip, as long as it is affected by an in-
fluence which emanates from the adjoining white yolk; but this abili-
ty manifests itself as a rule only in the region of the gray crescent,
to which it is due in the highest degree. Thus, as regards the power
of initiating the gastrulation process, there are only quantitative
differences in the surface layer of the egg. An embryonic anlage can
develop from each dorsal blastopore lip, once it has been laid down,
even from one which does not form in the region of the gray crescent.
Thus, when the groove was frontally located we found the first anlage
in some cases outside the region of the gray crescent, in the original
vegetal field (cf. for example, Figs. 57 a and 98 a) and, later, in
addition to the dorsal embryonic anlage, we also found a ventral one
(Figs. 57 b and 98 b). Hence, an influence inducing the remaining
embryonic material or a portion of it to form an embryonic anlage can
emanate not only from the dorsal region of the gray crescent, that is,
Spemann's organization center, but also from any other spot on the
embryo surface. Hence, our opinion (cf. Schleip and Penners 1926)
is that the dorsal region of the gray crescent, and presumably the
organization center of the amphibian in general, differs only to some
quantitative degree from the other parts of the egg surface.
Goerttler (1927) using another approach, has similarly come to the
opinion that within a dynamically conceived structure of the amphibian
embryo, an intensity maximum is present in the vicinity of the dorsal
blastopore lip and a declining intensity gradient extends from there.
And Vogt (Goerttler 1926) had already assumed that there are quanti-
tative differences, at least within the organization center itself.
Of course, our opinion does not preclude the possibility that at
later stages of development, the particular qualities of the organi-
zation center may not merely be of a quantitative nature.

      There is, as we have previously stressed (1926) a connection
between the results in the amphibians and a view which Boveri (1901)
presented in connection with the sea urchin: In this species, apart
from the entire animal region, any part of the blastula wall is
capable of mesenchyme and archenteron formation, but each part only   /151
forms that organ which it can do the best, and that is the "most
vegetal"; this then determines the role of the remaining parts in
the development. The "most vegetal part" of the sea urchin corres-
ponds, from the developmental-physiological standpoint, to the "most
dorsal region" of the amphibian egg and thereby, to the organization
center. Spemann (1903) has, besides, briefly referred to this
similarity between Boveri's and his own findings in his earlier ex-

periments, when he localized in the dorsal blastomere the necessary factor for the development of an embryonic anlage.

Considered from this standpoint, contrary to Vogt's (1926) view, there is, properly speaking, no reason why the amphibian egg should not be designated at least until the early cleavage stages, as a harmonious-equipotential system (Driesch 1921 and earlier). For some totality can develop from any part of its surface (which does not contain too much white yolk), namely, a blastopore anlage and thereby also an embryonic anlage. Only quantitative differences exist in this system, but Driesch does not exclude simple, polar differences from a harmonious-equipotential system either. According to Spemann (1927), the organization center in the later stages of the Urodela embryo behaves as a harmonious-equipotential system; our interpretation naturally includes the proposition that, just as the entire surface, the gray crescent also represents such a system at the stages which were examined by us.

6. The potential of the ventral blastomere

In conclusion, we turn once again to the initial question which induced us to undertake these investigations. If the groove is laid down frontally, the boundary between the dorsal and ventral half-blastomeres forms on the vegetal surface of the egg (or, if the first furrow ran in the median plane, the boundary between the two dorsal and the two ventral 1/4-blastomeres, which results in the same thing). In such cases, a complete individual can also develop from the ventral blastomere (or from both ventral cells), and even from it alone, while the dorsal cells do not form any individual (cf. the summary on p. /130). That can also occur if the groove was not laid down in the region of the original gray crescent but in the region of the original vegetal field. In such cases, the ventral half contains nothing of the region which, according to Spemann, and according to Bautzmann's more precise delimiting experiments (1926), represents the organization center. But it always contains the ventral ends (horns) of the gray crescent which, as Vogt (Goerttler 1926) presumed, possess organizing capacities in the sense indicated by Spemann, just as has its dorsal portion, only to a lesser degree than the latter. The capacity of the ventral blastomere for forming an embryonic anlage could be traced back to this circumstance; this is also indicated by the cases in which the frontally running furrow is first laid down in the vicinity of one or both horns of the gray crescent. This interpretation, however, cannot fit other embryos in which the frontal groove first appears in the region of the original vegetal field and the anlage of the

ventral individual thus evolves from a point which does not belong to the gray crescent.

Already in 1925 (p. /141) we reported on an experiment in which double formations developed from 53 embryos with frontal groove, and in these, one individual must have evolved from the ventral half of the egg. Numerous other cases of this sort are presented in Part V in more detail.

Our results contradict Spemann's view (1901-1903, 1914, 1918) that the ventral 1/2-blastomere (or the ventral half of the unfertilized egg) in Triton can gastrulate but cannot form any embryonic anlage. The possibility that Rana and Triton behave differently in this respect is not excluded but it is not very probable. But Spemann's view can also be demonstrated as inapplicable to Triton; it is rather only an interpretation of the finding that on the separation of the 1/2 blastomeres, either both of them yield an embryo or only one of them does while the second may gastrulate but does not form an embryonic anlage. Spemann accounted for this with two assumptions: First, the first cleavage is considered to run either in the median or in the frontal plane. This assumption is no longer tenable, for according to Vogt's investigations (1923 and later) the first cleavage can form any sort of angle to the median plane. Secondly, only the dorsal half of the egg (in the organization center) contains the factor necessary to the formation of an embryonic anlage. Consequently, if the first cleavage is in the median plane, each 1/2 blastomere contains a part of this factor; if it is frontal, the factor comes completely into the dorsal one. The latter assumption would only be demonstrated if one could definitely distinguish between the dorsal and the ventral blastomeres of the Triton egg -- by, say, the type of gray crescent (cf. Vogt 1923, 1926) -- and if it then turns out that the isolated ventral blastomere in no case forms an embryo. The fact that, on being isolated, sometimes the two first blastomeres in Triton form one embryo each and sometimes only one /153 does, can no longer serve as support for Spemann's assumption, since in inverted Rana eggs with a frontal groove, sometimes both halves produce an embryonic anlage, sometimes only the dorsal one does and sometimes only the ventral one does. The experiment in which the dorsal and the ventral embryo halves are first separated at the stage of incipient gastrulation, at which time they can be distinguished from one another on the dorsally situated blastopore lip, appears to support Spemann's view, however. For, in this experiment, the ventral gastrula half was incapable of forming an embryonic anlage (Spemann 1903, 1918, Ruud and Spemann 1922). But if the ventral half of the gastrula can produce an embryo up to the four- or eight-celled stage, this does not mean at all that it is still capable of such formation at the stage in which gastrulation has begun. During the first few cleavage stages, the egg can still be an equipotential system in the

above stated sense of the word without having to be such a system at a later time as well.

The ventral blastomere of the R. fusca egg, and presumably that of the other amphibians as well, is thus capable of forming a complete embryonic anlage. Brachet (1927) has, according to a brief note, come to the opposite conclusion for R. fusca, but in light of our positive results, his negative results are untenable.

The capacity of the ventral blastomere to form an entire embryonic anlage does not depend upon the cell's acquisition of the organization of a complete egg, as one could assume from the explanation of the Schultze experiment commonly offered up to now. Furthermore, in the inversion experiment, double formations do not generally develop when both 1/2 blastomeres contain the structure of an entire egg and are isolated physiologically from one another (cf. Part IV C 2). The ventral blastomere is, furthermore, capable of forming an embryonic anlage for reasons other than because such a form might be induced in it by the dorsal embryonic anlage, as Spemann presumed (1918) (cf. Part I, p. /321). This is because the effect on the ventral blastomere of an organization center which belongs to the dorsal blastomere alone, would not be explainable, according to all other experiences, at least in the case when the frontal groove develops outside the region of the organization center. The full potential of the ventral blastomere depends rather on other causes: Perhaps the frontal groove even is laid down within the gray crescent in particular cases and then the ventral blastomere contains a portion of the organization center. We have originally (1925, p. /144) taken this possibility for granted in the explanation of our results; it is not supported by the factual evidence however. It is certain, however, that the frontal groove can be laid down on the boundary between the middle part of the gray crescent and the original vegetal field or entirely within the latter. The margin of the groove belonging to the ventral part of the embryo functions like an organization center even though it contains nothing of the original organization center. This explanation ensues from our total findings concerning the causes for the development of the Schultze double formations.

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